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The Gut Microbiota as Target for Innovative Drug Development: Perspectives and a Case Study of Inflammatory Bowel Diseases

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1. Introduction

For many centuries scientists have been intrigued by the function of the intestine in human health and have tried to explain the role of the intestinal tract in our body, with sometimes rather original interpretations of its function. Leonardo Da Vinci for instance, who described the anatomy of the human body in great detail, concluded that the digestive system is in fact a part of the respiratory system, supporting its functioning. Indeed, he stated the following, giving a quite original interpretation on the function of the intestines: "The compressed intestines with the condensed air which is generated in them, thrust the diaphragm upwards; the diaphragm compresses the lungs and expresses the air" (O'Malley & Saunders, 1982). So gasses produced in the intestine would help to breath… In general, until quite recently it was believed that the main function of the intestine would be to dispose waste materials and reabsorb water from the intestine. Since the work of Antonie Van Leeuwenhoek it is known that the intestine contains an extensive microbial community (Smit & Heniger, 1975).

Nowadays, it's clear that the intestine is much more than an organ for waste material and absorption of water, salts and drugs, and indeed has a very important impact on human health, for a major part related to the specific composition of the complex microbial community in the colon. This microbial community composition is governed by age, diet, environment and phylogeny (Ley et al., 2008; Zoetendal et al., 1998; Zoetendal et al., 2001) and contains all three domains of life: Bacteria, Archaea and Eukarya (fungi, yeasts and protozoa). The human colon harbors a highly complex microbial ecosystem of about 200 g living cells, at concentrations of 10^{11} microorganisms per gram gut content, in total numbers which outnumber the amount of somatic and germ cells in the human body with a factor 10. Together, all 6.5 billion humans on earth represent a gut reservoir of 10^{23}-10^{24} microbial cells, which is just five orders of magnitude less than the world's oceans (10^{29} cells) (Ley et al., 2006). Therefore, the human gut constitutes a substantial habitat in our biosphere and we can in fact consider the human body as a mix of human and bacterial cells. Despite such high numbers, the microbial diversity is however relatively limited. Although 55 and 13 divisions of respectively Bacteria and Archaea have been described, only 8 bacterial

divisions have been identified so far in the gastrointestinal tract (GIT) and the gut microbiome is dominated by only 2 bacterial divisions, Firmicutes and Bacteroidetes, that make up over 90% of the intestinal microbiota. The remainder consists of Actinobacteria (Turnbaugh et al., 2009) and, to a lesser extent, Proteobacteria, Verrucomicrobia, and Cyanobacteria (Backhed et al., 2005; Eckburg et al., 2005; Ley et al., 2006). Further, only two archaeal species have been described with *Methanobrevibacter smithii* being more predominant than *Methanosphaera stadtmanae* (Eckburg et al., 2005). At this level, the intestinal communities of all humans therefore appear quite similar. However, within these divisions, a limited number of lineages terminate in broad, shallow radiations comprising hundreds of species and thousands of strains, making the microbiota of an individual as personalized as a fingerprint (Backhed et al., 2005; Ley et al., 2006).

In addition to the very high numbers of microbes in our intestines, it is estimated that the collection of all microbial genomes comprises 2 to 4 million genes, which is 70 to 140 times more than that of their host. This 'microbiome' encompasses all genes that are responsible for numerous processes such as substrate breakdown, protein synthesis, biomass production, production of signaling molecules, antimicrobial compounds and encodes biochemical pathways that humans have not evolved (Egert et al., 2006). The microbiota in the large intestine can therefore be seen as a separate organ encompassing a broad range of specific additional activities, which provide great opportunities for its host and which is capable of even more conversions than the human liver.

Summarized, the intestinal microbial ecosystem not only constitutes a major part of the body's cell numbers, its extensive gene pool represents an almost unlimited functionality in our body. Whereas typical examples of such activity relate to the extraction of energy from otherwise indigestible plant polysaccharides, it has become clear that the mutualistic interaction between the host and its microbiota, has a major influence on the host's health. This includes the stimulation of the gut immune system (Salminen et al., 1998), the regulation of cell proliferation (Dethlefsen et al., 2006), the synthesis of essential vitamins K and B (Conly & Stein, 1992), and providing resistance to colonization by pathogens (Hopkins & Macfarlane, 2003). However, recent insights in the composition of the intestinal microbiota and host-microbiota cross-talk have shown that the balance in this mutualistic interaction is very fragile and a dysregulation in specific community assemblages is now considered as a risk factor contributing to a disease state. This is shown by recent reports linking intestinal bacteria with diseases ranging from allergies (MacDonald & Monteleone, 2005) to bowel inflammation (Elson et al., 2006), and obesity (Backhed et al., 2007).

If we better understand the possibilities of the intestinal microbiome and the importance of optimal host-microbiota cross-talk, this will off course open up completely new perspectives to exploit this role of the microbiota to improve host health and for innovative drug development (Possemiers et al., 2009). Into a major extent, such strategies can relate to the potent, almost unlimited metabolic potential of the intestinal microbiome. Whereas metabolic processes in the body which affect the final bioactivity of drug compounds have typically been associated with the hepatic metabolism, microbial drug biotransformation may also dramatically affect drug bioavailability and a possible lack thereof (Possemiers et al., 2011a). As current evaluation of drug bioavailability and biological activity is typically based on the ADME principle (Absorption, Distribution, Metabolism and Excretion), inclusion of microbial metabolic processes in drug bioavailability screening should indeed become standard practice. There is therefore a clear need to better understand the microbiota and its metabolic capability, which will be discussed in this book chapter.

Drug development may reach far beyond only understanding intestinal metabolic processes. Indeed, innovative strategies which actively make use of bacterial cells and their metabolic functionalities to improve drug bioavailability have been developed over the last decade. Several specific examples will be provided in the book chapter, ranging from the use of intestinal microbial metabolism for targeted prodrug activation up to the use of microorganisms as production facilities inside the intestine for innovative drug development.

An important challenge towards such new drug development strategies is the high complexity of intestinal (microbial) processes. Not only is the lack of direct accessibility to study intestinal processes in the gut itself a strong limitation for using animals or humans in the screening process, the intestinal microbiota is also characterized by a dramatic interindividual variability, ultimately leading to a strongly varying response between individuals. This interindividual variability together with the need for a suitable environment for mechanistic mode-of-action studies create the necessity for suitable *in vitro* and *in silico* models to predict metabolic fates of drugs. Complex multi-stage *in vitro* models together with *in silico* models can provide us with very valuable information and will therefore also be discussed in this chapter.

Finally, several of these aspects will be handled with inflammatory bowel diseases (IBD) as case study, a set of inflammatory diseases in the intestine for which a bacterial role has been described.

2. The microbial metabolic perspective

A wide range of metabolic reactions can be catalyzed by the large diversity of microbial enzymes. Ilett et al. (1990) have already indicated that gut bacterial metabolism is reductive, hydrolytic and even of degradative nature with a strong potential for both bioactivation and detoxification of xenobiotics. The latter is in contrast to the oxidative and conjugative reactions from the phase I and II enzymes in the enterocytes and hepatocytes (Ilett et al., 1990). Moreover, the intestinal microbiota interferes with the human biotransformation process through the enterohepatic circulation of xenobiotic compounds what may reverse the detoxification cycle of the liver. Additionally, the microbial metabolic potency can be addressed for the local release of the active compounds in the colon and as production facility for specific active compounds.

Figure 1 gives a schematic overview of the microbial metabolic potential and its interference in the enterohepatic circulation and Table 2 gives an overview of the described drug development approaches making use of bacterial cells and their metabolic functionalities throughout the book chapter.

2.1 Direct microbial metabolism influences final activity profiles

Chemical components in food, either as food component or as contaminant, represent a significant source of both positive and negative influences on the health and function of the human body. For example, polyphenol mixtures represent one such class and within a much wider realm of application, there is now substantial evidence to support the hypothesis that these may be beneficial in the prevention of cardiovascular diseases, cancers, neurodegenerative diseases, diabetes, or osteoporosis risk (Scalbert et al., 2005). It is now well investigated that the majority of polyphenol compounds reach the colon unaltered. There, they undergo extensive metabolism by the resident gut microbiome prior to

absorption. This is a key step in understanding the fate of many xenobiotic compounds and their final clinical effects and yet this process has remained almost completely unexplored.

An important field of research dealing with gut microbial conversion of food compounds is that of a specific group of polyphenols: the phytoestrogens. Phytoestrogens are plant components that structurally resemble 17β-estradiol. Depending on the target organ and the endogenous estrogen levels, these phytoestrogens may elicit an estrogenic or anti-estrogenic effect, by which they may play a role in the prevention and treatment of hormone-dependent diseases such as breast- and prostate cancer, or osteoporosis and menopausal complaints. The most important classes from nutrition are isoflavones (primarily from soy), coumestans (fruit, cereals, leguminosae), prenylflavonoids (hops), and lignans (fruit, cereals, leguminosae). Hop contains the most powerful phytoestrogen known thus far, namely 8-prenylnaringenin (8-PN). Rowland et al. (2003) discussed the role of the gut microbial community in the bioavailability and metabolism of estrogens in general and phytoestrogens more in particular. Intestinal bacteria contribute to the bioavailability and biological activity of phytoestrogens in a number of ways. Firstly, intestinal bacteria produce β-glucosidases thereby cleaving the naturally occurring glycosylated phytoestrogens into an aglycon. Secondly, the gut bacteria elicit glucuronidase and sulfatase activity, thereby deconjugating phase II metabolites from the liver and releasing the aglycons again. Thirdly and most importantly, intestinal bacteria are capable of transforming the original components to metabolites that have a higher biological activity. For example, equol and 8-PN are bacterial metabolites from daidzein (soy) and isoxanthohumol (hop), respectively and have a much higher biological activity than their precursor product. A remarkable aspect of the microbial conversion of daidzein into equol and of isoxanthohumol into 8-PN is the large interindividual variability in the conversion efficiency. This was illustrated for equol production (Bolca et al., 2007a) and 8-PN production (Bolca et al., 2007b).

A second example of a natural product used for its medicinal properties are anthranoid-containing laxatives. Anthranoids are obtained from the dried leaflets and pods of plants such as senna plants, *Cascara sagrada*, *Frangulae cortex* and rhubarb. In plants, anthranoids are mostly present as sugar derivatives and due to this β-glycosidic linkage the molecule is carried unabsorbed into the large intestine where the microbial metabolism starts and the active aglycon anthrone is released (de Witte & Lemli, 1990). Changes in the colonic motility, absorption, and secretion result in an increased intestinal transit rate and fluid accumulation. Chronic use of these laxatives causes melanosis coli, a deep black pigmentation of the colon mucosa but whether there is an association with colorectal cancer, is still controversial (Van Gorkom et al., 1999; Nusko et al., 2000).

Besides the interest in the microbial conversion of health-promoting components from ingested foodstuffs, much attention is also given to the role of intestinal bacteria in the conversion of ingested drugs, environmental or food contaminants or xenobiotics in general. The ability of the gut bacteria to metabolize drugs came under the attention of pharmaceutical companies since an accident in 1993 with the antiviral drug orivudine which revealed that its gut microbial metabolite (E)-5-(2-bromovinyl)uracil interfered with the clearance of a co-administered anti-cancer drug 5-fluoro-uracil. This resulted in the death of 18 patients (Okuda et al., 1998). Recently, Sousa et al. (2008) reviewed the conversion of over 30 drug compounds by gut microorganisms and the related consequences for their biological effect in the human body. Examples are the reduction of omeprazole and digoxin, hydrolysis of lactulose and sorivudine, acetylation of 5-aminosalicylic acid, proteolysis of

insulin and calcitonin and N-demethylation of methamphetamine. Additionally, demethylation, deamination, decarboxylation and dehalogenation reactions have also been described, next to other reactions such as aromatization, esterification and N-nitrosation (Ilett et al., 1990). Microbial metabolism is not only confined to drugs, but also targets contaminants. The heterocyclic aromatic amine, 2-amino-3-methylimidazo[4,5-f]quinoline (IQ) – a compound which is produced during the cooking and grilling of meat – is converted by intestinal bacteria into the direct acting mutagen 7-hydroxy-IQ (van Tassell et al., 1989). IQ incubation studies of intestinal microbiota suspensions from different individuals showed a large interindividual variability between the potency to form the 7-OH-IQ. Apparently, this reaction can be carried out by bacteria belonging to the predominant populations of the human gut, such as *Bacteroides thetaiotaomicron*, *Clostridium clostridiiforme*, and *Escherichia coli* (Humblot et al., 2005). As a third example of microbial metabolism, oxidative reactions were reported by Van de Wiele et al. (2005) who described the hydroxylation of polycyclic aromatic hydrocarbons (PAHs) by *in vitro* cultured intestinal microbiota. Hydroxylation of PAHs gives these compounds an affinity for the human estrogen receptor. In that way, hydroxylated PAHs may act as pseudo-estrogens and interfere with normal hormone-driven process in the body. A final example targets the conversion of metal(loid)s, both in an inorganic as an organic chemical form. Michalke et al. (2008) reported that human gut microbes actively volatilize bismuth and other metal(loid)s, including arsenic (As), through methylation and hydrogenation. Moreover, Meyer et al. (2008) postulated that gut methanogens play a crucial role in metal(loid) volatilization, thereby exerting toxic effects to the human body, not only by direct interaction with the host but also by disturbing the endogenous gut microbiota composition and metabolism. Further, a thorough *in vitro* exploration with the Simulator of the Human Intestinal Microbial Ecosystem (SHIME®), a dynamic human gastrointestinal simulator, revealed a high microbial metabolic potency toward metal(loid)s (Diaz-Bone & Van de Wiele, 2009). This was demonstrated by the finding of significant volatilization of arsenic (As), selenium (Se), bismuth (Bi), tellurium (Te), and antimony; the formation of highly toxic AsH_3 (arsine) and $(CH3)_2Te$ (dimethyl telluride); and the discovery of two new As–sulfur metabolites. Van de Wiele et al. (2010) and Pinyayev et al. (2011) later showed the contribution of the gut microbiota, presumable sulfate reducers, in the formation of methylated thioarsenicals from which the toxicity is thought to be equivalent to that of trivalent inorganic arsenic.

2.2 Microbial metabolism interferes with the enterohepatic circulation

Compounds that have been absorbed in the intestine and subsequently detoxified are usually conjugated with polar groups (glucuronic acid, glycine, sulfate, glutathion, and taurine) in the liver prior to secretion with the bile (Ilett et al., 1990). After released in the intestinal lumen, these phase II biotransformation products may be hydrolyzed again by bacterial enzymes such as β-glucuronidase, sulfatase, and other glycosidases. This would negate the detoxification cycle and delay the excretion of many exogenous compounds since the original compounds or phase I metabolites are more prone to intestinal absorption than their phase II conjugates. Several studies have already shown the biotransformation capacity of gut bacteria towards hydrocarbons. For example, bacterial β-glucuronidase activity hydrolyzes the glucuronidated form of IQ, a food-borne carcinogen, thereby increasing the colonic genotoxicity of this compound in rats (Humblot et al., 2007). Additionally, guinea pig experiments showed that the hydrolysis of benzo(a)pyrene conjugates by the intestinal

microbiota resulted in *de novo* production of toxic benzo(a)pyrene intermediates what caused the formation of DNA adducts in the colon (Bowes & Renwick, 1986). Other research studies have described similar deconjugation reactions for many other compounds.

2.3 Colon targeted drug release using the microbial metabolism

The enormous metabolic potency from the gut microbiota can also be employed for colon-targeted drug delivery. The targeted delivery of drugs in the colon provides certain benefits, or is even necessary in certain therapeutic scenarios. Depending on the nature of the administered molecule, drugs can be highly sensitive to the stringent conditions of the upper GIT (low gastric pH, high enzymatic activity), which makes it necessary to postpone their intestinal absorption to the lower GIT (distal ileum or colon). In addition, patients that suffer from diseases that are more confined to the colon region (f. ex. IBD, carcinoma or bacterial infection) may benefit from a local rather than a systemic therapy. Such local drug release would optimize the therapeutic efficiency or minimize possible side effects from a systemic (intravenous) drug administration. Two strategies can be used to involve gut microorganisms.

A first strategy makes use of prodrugs, which are pharmacologically inactive derivatives of the parent drug compound (reviewed by Patel et al., 2007). These prodrugs are designed to be more resistant to breakdown processes in the upper digestive tract (f. ex. acid hydrolysis, protease activity) and require conversion to the bioactive molecule by enzymatic activity. This bioactivation process often relies on gut microbial enzymatic activity, which dramatically increases due to the high microbial load in the colon environment (10^{10}-10^{11} microorganisms per gram versus 10^{6}-10^{7} microorganisms per gram in the ileum). Azo-bond prodrugs and glycoside prodrugs are considered the most important classes of prodrugs, while glucuronate, dextran, cyclodextrin and polypeptide prodrugs are also manufactured (Patel et al., 2007).

A second strategy that requires gut microbial activity to deliver drugs in the colon is the coating of drugs with biodegradable polymers. In this case, the polymer is resistant to the digestive processes from the upper GIT and thereby protects the encapsulated drug molecule. The polymer is however subjected to microbial biodegradation in the colon and subsequently releases the drug molecules, which can then be taken up across the colon epithelium. To illustrate, azo-containing polyurethane molecules have been used as a coating material for drugs. The molecule is degraded by gut microbial azoreductase activity and subsequently releases the active drug compound (Chavan et al., 2001; Kimura et al., 1992).

Colon-specific drug delivery systems making use of these microbial properties have been successfully applied for both local as systemic drug therapy (Patel et al., 2007).

2.4 Bacteria as production facilities

Bacteria may furthermore be used as actual 'production facilities' that produce active compounds. The most well-known example is probably the production of antibiotics by microorganisms. Due to the selection of resistant pathogens, there is a contuining need for new antibiotics. This need has been largely met by the production of synthetic antibiotics but due to advances in technology there is an increasing interest in the production of natural antibiotics from bacteria. Soil actinomycetes have been a major source of

antibiotics and recently, searches of underexplored ecological niches (f. ex. deep-sea sediment samples, bacterial symbionts of insects or fungi, Mycobacteria) have revealed new molecules. These molecules produced by the bacteria are essential to combat resistant pathogens, which are increasingly prevalent in the community (Clardy et al., 2006; Fischbach & Walsh, 2009).

As a second example, we will discuss here in detail the case of carotenoids in which *Bacillus* spores are used as 'production facilities' producing active ingredients directly in the intestine. Mammals cannot synthesize carotenoids *de novo* and must acquire them from the diet. These are key components that have a role in nutrition (Vitamin A), vision and its development (retinoic acid). Moreover, the antioxidant properties of carotenoids protect the cells from environmental stresses and are also able to prevent the onset of chronic disease states in mammals (Duc et al., 2006; Giovannucci, 2002; Mares-Perlman et al., 2002). In order to exert these beneficial effects in the GIT, ingested carotenoids must reach a concentration that is sufficient to act as a scavenger of oxygen radicals (Agarwal & Rao, 1998; Fuhrman et al., 1997; Witztum, 1994). Finally, after intestinal absorption, carotenoids can also have anti-inflammatory or anti-carcinogenic effects (Ben-Dor et al., 2005). The problem is that pure carotenoids are rapidly degraded in the stomach. It is possible to increase their resistance by incorporating high doses of carotenoids into a food matrix in order to guarantee a recommended daily intake of about 800 $mg.day^{-1}$. However, this enrichment procedure is expensive and therefore limited (Duc et al., 2006). Recently, scientists at the Royal Holloway University of London have isolated carotenoid-producing spore-forming *Bacillus* spp.. These pigmented *Bacillus* species (yellow, pink, orange…) have been characterized and the pigments have been shown to be due to one or more carotenoids (Duc et al., 2006; Hong et al., 2009; Khaneja et al., 2010; Perez-Fons et al., 2011). More specifically, the presence of 1-HO-demethylspheroidene, ubiquinone and phytoene was identified in some strains by means of a combination of HPLC analysis and UV/VIS spectral data. The carotenoids contained within the spores appeared to be gastric stable and could therefore provide a good source of carotenoids in the small intestine where - following the germination of the *Bacillus* strains - they are released. The released carotenoids or their metabolites may be absorbed from the gut and reach systemic circulation. Alternatively, a part of the carotenoids and their derivatives may reach the colon, where they can be subjected to further microbial metabolism and/or absorbed through the colonic epithelium. This is particularly appropriate for the carotenoids still contained in the spores, as the release before the beginning of the colon may not be complete. Moreover, if the bacteria are able to survive and grow in the colonic microbial community, they may produce and release additional carotenoids into the colonic environment. It has been shown that the *Bacillus*-derived apocarotenoids antioxidant properties are 10 times higher than lycopene. Next to acting as an optimal carrier for the improved delivery of gastric-stable carotenoids, the *Bacillus* strains may exert probiotic properties in the colon, resulting for instance in an improvement of the gastrointestinal environment, immune stimulation, antimicrobial activities and competitive exclusion (Cutting, 2011). A final added value is that, compared to other vegetative bacteria which are typically used as ingredient/probiotic, *Bacillus* spores are particularly attractive because they can be stored at ambient temperature in liquid or dried form indefinitely without the need for refrigeration, thereby ensuring better control of the administered doses.

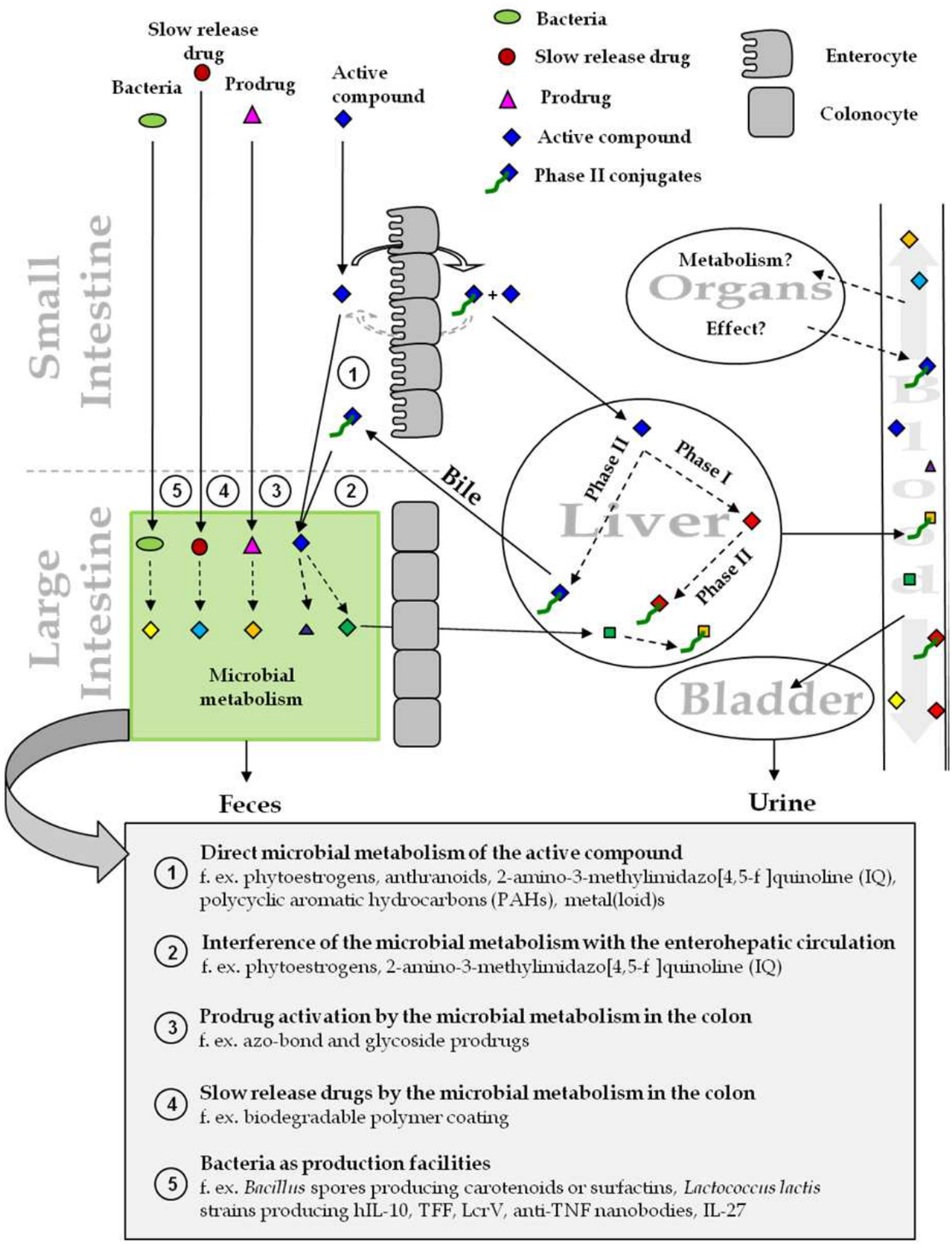

Fig. 1. Schematic overview of the microbial metabolic potential and its interference with the enterohepatic circulation. Adapted from Possemiers et al. (2011a)

3. Gut models for studying microbial modulation of human health

As mentioned in the previous paragraphs, the high complexity of intestinal microbial processes is a main challenge in new drug development strategies. There is a clear necessity of creating suitable *in vitro* and *in silico* models to predict metabolic fate of drugs. These models need to take into account the dramatic interindividual variability of the gut microbiota (both in terms of composition and activity) that ultimately leads to a strongly varying response between individuals. When using animals or humans in the screening processes, the lack of direct accessibility to study intestinal processes in the gut itself represents a serious obstacle to elucidate the importance of intestinal processes for a specific drug candidate. Moreover, whereas final *in vivo* testing is off course required to confirm formulation performance, this approach holds a number of drawbacks to be used in early stages of drug development such as ethical concerns, lack of information on the mechanism of action, analytical difficulties related to dilution of the active compounds and their metabolites in the plasma and the rest of the body as well as doubts on the representativeness of animal models for the human situation (Pieper & Bertau, 2010).

In vitro simulation studies may offer many unique advantages, even if they suffer from the absence of a complete physiological environment. They are easy to set up and sample, and have a high reproducibility. They offer the possibility of performing mechanistic studies and there are no ethical constraints. However, it is clearly of key importance to evaluate the specific properties of a candidate active compound under conditions that are relevant to *in vivo* situations. In fact, the better an *in vitro* system can simulate the real gut situation, the higher is the physiological significance of the obtained information (Marzorati et al., 2009).

In this respect, simple batch experiments, single stage reactors or the conventional dissolution systems do not replicate the rapidly changing dynamic environment of the gut lumen and the physiological processes occurring therein. In the last decade, the need of having systems that could better simulate *in vivo* conditions led to the creation and perfectioning of dynamic *in vitro* simulators that attempt to reproduce all or part of the physiological parameters that could influence the gastrointestinal microbial community and its metabolic activity (Macfarlane & Dillon, 2007; Minekus et al., 1999; Molly et al., 1993). These systems should allow recreating *in vivo*-like conditions in relation to the different sections of the GIT, i.e. stomach (secretion of gastric juice and simulation of fasted and fed condition); small intestine (secretion of pancreatic juice and bile salts, absorption of nutrients/electrolytes and high shear forces); large intestine (presence of a representative microbial community – in terms of activity and composition – in the ascending, transverse and descending colon); and finally, host-microbiota interaction (simulation of the final effect on the host).

Among the several options available on the market, the TIM model (TNO, Delft, The Netherlands) and the SHIME® (ProDigest and Ghent University, Gent, Belgium) are considered as being the most accurate imitations of the GIT both on a structural and a functional level (Pieper & Bertau, 2010).

The TIM model is composed of two separate computer-controlled units - TIM 1 and 2 - running independently (Minekus & Havenaar, 1996; Minekus et al., 1999). The TIM 1 system mimics the stomach and small intestine (i.e. duodenum, jejunum and ileum), while the TIM 2 is a simulation of the proximal colon of monogastric animals (pH = 5.8). Fluid transportation from vessel to vessel is executed by peristaltic valve-pumps and there is a constant absorption of water and of small lipophilic and hydrophilic compounds by means

of hollow fiber membranes. This system can be used to study drug-nutrient interactions, molecule bioconversion and nutrient compound bioavailability (Anson et al., 2009b; Blanquet et al., 2003). For example, the bioavailability of ferulic acid, an antioxidant, was studied with the TIM 1 system. It was found to be very variable and dependent on the food source (bran, flour, aleurone). However, supplementation of free ferulic acid to flour significantly increased its bioavailability. The authors concluded that the TIM model was a valid model to predict the *in vivo* bioavailability of ferulic acid (Anson et al., 2009b). In a TIM 2 simulation, the model is first inoculated with a frozen-conserved cell culture derived from a fecal inoculum. During the experiment, the system is fed with a 'Standard Ileal Efflux Medium' and samples can be taken both from the lumen of the simulator and from the dialyzed liquid during the simulation. It was shown by the TIM 2 model that bioprocessing of wheat bran (fermentation treatment or enzymatic- in combination with fermentation treatment) improves the colonic metabolism of ferulic acid (Anson et al., 2009a).

The prototype of the SHIME® was originally developed by Molly et al. (1993) (Figure 2). Nowadays, it is a computer-controlled system consisting of a succession of five reactors representing the complete GIT of the adult humans. The first two reactors are of the fill-and-draw principle and simulate the physiological processing occurring in the stomach and in the small intestine (i.e. different sigmoidal decrease of pH under fasted or fed conditions; addition of gastric enzymes, pancreatic and bile liquid). A dialysis filter is used to simulate the absorptive processes occurring in this area of the GIT. The last three compartments are continuously stirred reactors inoculated with a fresh dilution of a fecal sample (the characteristics of the donor can be decided according to the aim of the study). Retention time and pH of the different vessels are chosen in order to resemble *in vivo* conditions in the different parts of the GIT (pH in the range 5.6-5.9, 6.2-6.5 and 6.6-6.9 in the ascending, transverse and descending colon, respectively) (Possemiers et al., 2004). No absorption is simulated in the large intestine. In a TWINSHIME®, in which 2 systems are run in parallel, long-term (up to 3 weeks) placebo-controlled *in vitro* studies or direct comparison of two different treatments are possible without interference of external parameters (Grootaert et al., 2009; Marzorati et al., 2009; Possemiers et al., 2008). The possibility of performing long-term studies with the SHIME® is of special interest. In fact, it has to be taken into account that bacteria may need to adapt their metabolism in order to be able to degrade a specific active compound. In this respect, a study conducted with a single dose of the active product may results in a wrong interpretation of the possible effect of the microbial metabolism on the product itself. These long-term studies are of special relevance when investigating the chronic exposure to a given compound. The SHIME® model was found to be an ideal model to study the bioavailability of isoxanthohumol, a phytoestrogen, allowing to study the microbial metabolism in the different parts of the intestine (Possemiers et al., 2006). Moreover, using the TWINSHIME® model, the probiotic effect of the 8-PN producing strain *Eubacterium limosum* could be compared for high and low 8-PN producing individuals (Possemiers et al., 2008).

Whereas the TIM system appears nowadays as the most suitable simulator for the processes occurring in the upper GIT, the SHIME® can provide more reliable results in terms of simulation of processes at the level of microbial metabolism.

It has to be acknowledged that the simulation of absorption, which takes into account only diffusion, is limited in both systems, as well as the absence of a direct prediction of the possible effect of the treatment on the host (Pieper & Bertau, 2010). The coupling of these dynamic simulators with an "off-line" test system that makes use of Caco-2/HT 29 cell lines

has been proposed as a possible approach to increase the scientific outcome of the *in vitro* simulation (Deat et al., 2009; Possemiers et al., 2011a). Indeed, cellular models are a complementary tool to mimic the active uptake of active compounds and their metabolites. Moreover, the exposure of these cells to the complete luminal content of the GIT, containing both the active compound, its potential metabolites and the rest of the intestinal environment, creates a situation closer to the *in vivo* condition as compared to those studies where the cells are only exposed to the active compound as a pure product. This approach was recently used to study the immune modulating properties of a dried fermentate derived from *Saccharomyces cerevisiae*. The SHIME® experiment confirmed quantitative increases in lactobacilli, qualitative modulation of both general and specific populations, reduction of pathogens, and even showed an increase in the production of the immune-protective short-chain fatty acid, butyrate. Moreover, treatment of Caco-2 cell lines with the intestinal suspension significantly decreased the production of the pro-inflammatory cytokine IL-8 (Possemiers et al., 2011b).

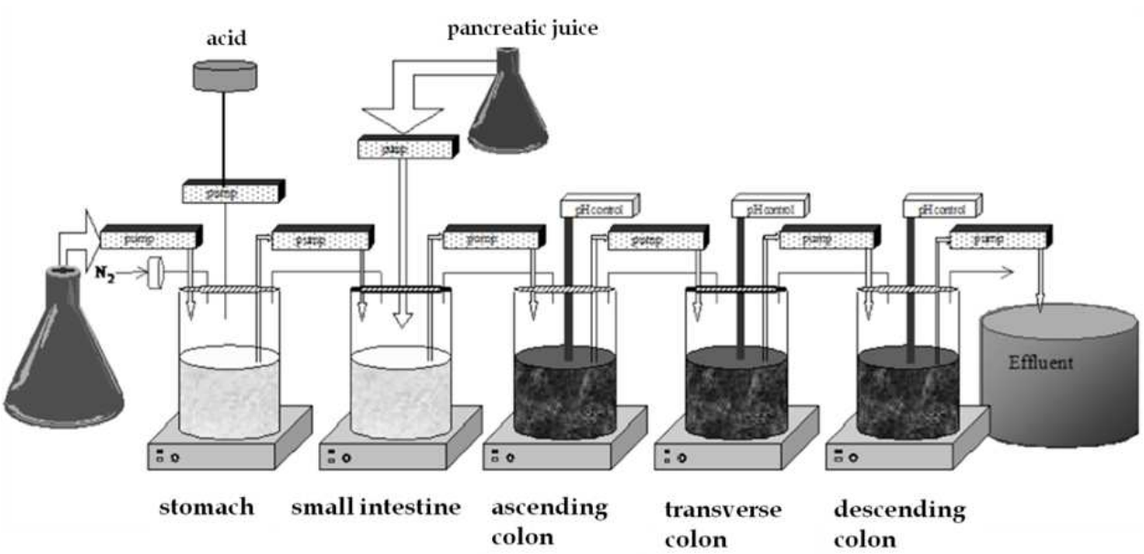

Fig. 2. Scheme of the Simulator of the Human Intestinal Microbial Ecosystem (SHIME®)

As compared to the state of the art, two further developments have been recently conducted in order to improve the simulation power of the SHIME®: the M-SHIME (Mucus-SHIME) and the HMI (Host-Microbiota Interaction) module. In the so-called M-SHIME, a mucosal compartment (mucin-covered microcosms) has been introduced in each colon vessel in order to reproduce the bacterial adhesion to the gut wall mucus layer (Van den Abbeele et al., 2011). This improvement allows evaluating the colonization and development of specific microorganisms that benefit from mucosal adhesion, microorganisms that would otherwise be washed out in those systems that only simulate the gut lumen. These are those microorganisms whose metabolism can have profound health effects in consideration of the fact they live in strict contact with the host surfaces.

Finally, the HMI module is a two-compartment model which allows to investigate in one anaerobic compartment the development of the mucosa-associated microbiota under realistic conditions of shear stress and to culture eukaryotic cells in the lower aerobic compartment for up to 48 h (Marzorati, 2010). The two compartments are separated by a

semi-permeable membrane that allows to simulate oxygen diffusion (micro-aerophilic conditions at the base of the biofilm) and bi-directional transport of molecules (i.e. absorption of microbial metabolites and excretion of host defence molecules). This new module has been tested in combination with the above-mentioned SHIME® in order to perform 'on-line' continuous experiments but, in principle, it can also be combined with other GIT simulators available on the market (Marzorati et al., 2011). Also in this model, the dried *Saccharomyces cerevisiae* fermentate was found to have immunomodulatory effects by decreasing the production of pro-inflammatory compounds, IL-8 and IL-1β (Marzorati et al., 2011). At the moment the system is conceived to evaluate the effect of microbial processes on the host cells and the effect of host cells on microbial processes. However, a simple addition of a third compartment would provide also the possibility of performing studies of bioavailability through cell lines (Figure 3).

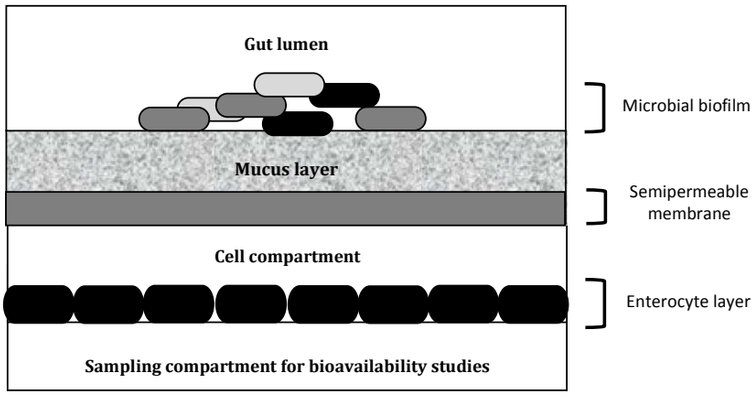

Fig. 3. Scheme of the Host-Microbiota Interaction (HMI) module. Adapted from Marzorati et al. (2011)

Table 1 summarizes the main characteristics of the two GIT simulators with respect to: i) the possibility of performing studies related to new drug development, and ii) the possibility to investigate the role of the microbial metabolism on the biotransformation of active compounds.

Another tool that can be useful in the cost-intensive process of a new drug development is the use of *in silico* models for biosimulation. These are normally considered as an alternative to the classical *in vivo* and *in vitro* studies. They make use of mathematical models to assess drug absorption, distribution, metabolism and excretion (Pieper & Bertau, 2010). The most commonly used models are the physiologically-based pharmacokinetics (Leahy, 2003), the systems biology-based drug metabolism simulation (Bugrim et al., 2004), the quantitative structure-activity relationship modelling (Chang & Swaan, 2006), and the computational oral absorption simulation (Sugano, 2009). More specific information on the topic has been recently reviewed by Bertau et al. (2008) and Pieper & Bertau (2010). A common trait of all these models is the fact they mainly focus on (hepatic) host metabolism, thereby omitting the role of the gut microbiota due to its extreme complexity and the lack of knowledge related to several metabolic processes occurring in the GIT. Even if the potential role of bacteria in metabolizing the active compound into other intermediates of degradation – that could have a deleterious effect for the human health or simply hinder the efficacy of the product itself – is clearly acknowledged, it is still not possible to model the complex

bacterial network within the gut. Currently on-going metagenomic and metatranscriptomic studies will provide us with further information with respect to this topic and, in a later stage, it will be possible to complement the already available models with a new set of data that will allow a simulation of the processes closer to the reality.

Characteristic	TIM	SHIME®
Simulation of the upper GIT	Yes	Yes
Simulation of the lower GIT	Yes	Yes
Full GIT in a single system	No	Yes
Simulation of the different regions of the small intestine	Yes	No[1]
Simulation of the different regions of the colon	No	Yes
Fecal inoculum	Frozen culture	Fresh inoculum[2]
Peristaltic movement	Yes	No[3]
Simulation of the absorption in the colon	Yes	No
Possibility of performing bioavailability studies (passive diffusion)	Yes[4]	Yes
Possibility of using the fluid from the system on Caco-2 cell lines to study active transport	Yes	Yes
Possibility of combining the simulator with the HMI module	No	Yes
Possibility of performing long-term studies[5]	No	Yes

[1] The pH increase along the small intestine can be simulated during the incubation
[2] Possibility of choosing a donor with specific characteristics
[3] Magnetic stirring
[4] TIM1 was specifically designed for this purpose
[5] To specifically follow up the adaptation of the microbial metabolism to the active compound

Table 1. Comparison of the TIM and SHIME® model

4. Case study: The gastrointestinal microbiota and inflammatory bowel diseases

In the next paragraph, the importance of the gut microbiome and its metabolism in drug development will be further discussed by means of a specific case study, i.e. IBD. The microbial metabolic potential as a therapy for IBD has been exploited and has provided a successful and widely used treatment for IBD which will be futher discussed in detail. Moreover, as no cure is available, there is an ungoing search for new therapies with the microbial potential as a very interesting target and tool.

4.1 The role of gut bacteria in immune homeostasis

Optimal immune functioning is crucial for the health and normal performance of humans and animals. The immune system operates at the systemic as well as at the local level, the latter which includes the mucosal tissue in the gut and upper respiratory tract. Whereas the systemic

immune system protects us from infections and disease in general, mucosal immunity has the important function of first line defence against penetrating allergens and pathogens.

Due to its unique function, about 70% of the body's immune system is found in the digestive tract. Indeed, the GIT is the site where the divergent needs of nutrient absorption and host defence collide. Whereas nutrient absorption requires a large surface area and thin epithelium, such design has the potential to compromise host defence. The body therefore needs an extensive immune protection in the gut to counteract this potential threat. The immune system of the gut divides into two parts, the physical barrier of the intestine and the active immune components. The physical barrier is central to the protection of the body to infections and the excessive penetration of allergens. Acid in the stomach, active peristalsis, mucus secretion and the tightly connected monolayer of the epithelium each play a major role in preventing microorganisms from entering the body. The cells of the immune system are organized in a complex pattern within the intestine, i.e. the gut-associated lymphoid tissue (GALT) (Gaskins, 1997). GALT comprises cells from both the innate and adaptive immune system. The innate immunity is responsible for the recognition of endogenous microorganisms, which is essential to maintain intestinal immune homeostasis. A schematic overview of the recognition, activation and response of the innate immune system is shown in Figure 4. Innate immune recognition is based on the detection of molecular structures that are unique to both pathogenic and non-pathogenic microorganisms, called microbe-associated molecular patterns (MAMPs), like lipopolysaccharide (LPS) or lipoteichoic acids (LTA) (Medzhitov, 2007). The main classes for the detection of MAMPs are pattern-recognition receptors (PRRs) including transmembrane Toll-like receptors (TLRs) and cytosolic nucleotide-binding oligomerization domain (NOD) like receptors (NLRs) (Kelly et al., 2005). GALT has the constant challenge of responding to pathogens, while remaining relatively unresponsive to food antigens and commensal bacteria, the normal inhabitants of the gut (Sanderson & Walker, 2007). After the detection of the ligands, intracellular signaling results in the transcription of pro-inflammatory cytokines (Kawai & Akira). In response, the production of antimicrobial peptides (Salzman et al., 2010), tight junctions associated proteins (Su et al., 2009) and mucus that forms a protective polysaccharide glycocalyx bilayer on top of the epithelial cells (Johansson et al., 2008) is induced. While maintaining the capacity to eliminate infection and induce proper tissue repair, the host has to activate specific negative regulators and regulatory pathways to reduce the response to tissue injury.

Besides the activation of antimicrobial defence processes, the innate immune system can stimulate the adaptive immune response. The latter is activated by antigens that cross the epithelial barrier and are engulfed by antigen presenting cells (APCs) resulting in an antibody-mediated or cell-mediated immune response. The receptors on the immune cells provide a system by which infections with the same pathogen are remembered. This so called long-term memory is characteristic for the adaptive immune system (Carroll, 2004; Cooper & Alder, 2006).

Suboptimal functioning of the immune system in the gut may have important consequences for the gut environment itself (overstimulation may lead to excessive inflammation and inflammatory bowel diseases, whereas insufficient activity opens the way for pathogen infections) but may also affect the rest of the body (pathogen translocation or leakage of bacterial fragments from the gut into the blood may cause both acute and chronic inflammation).

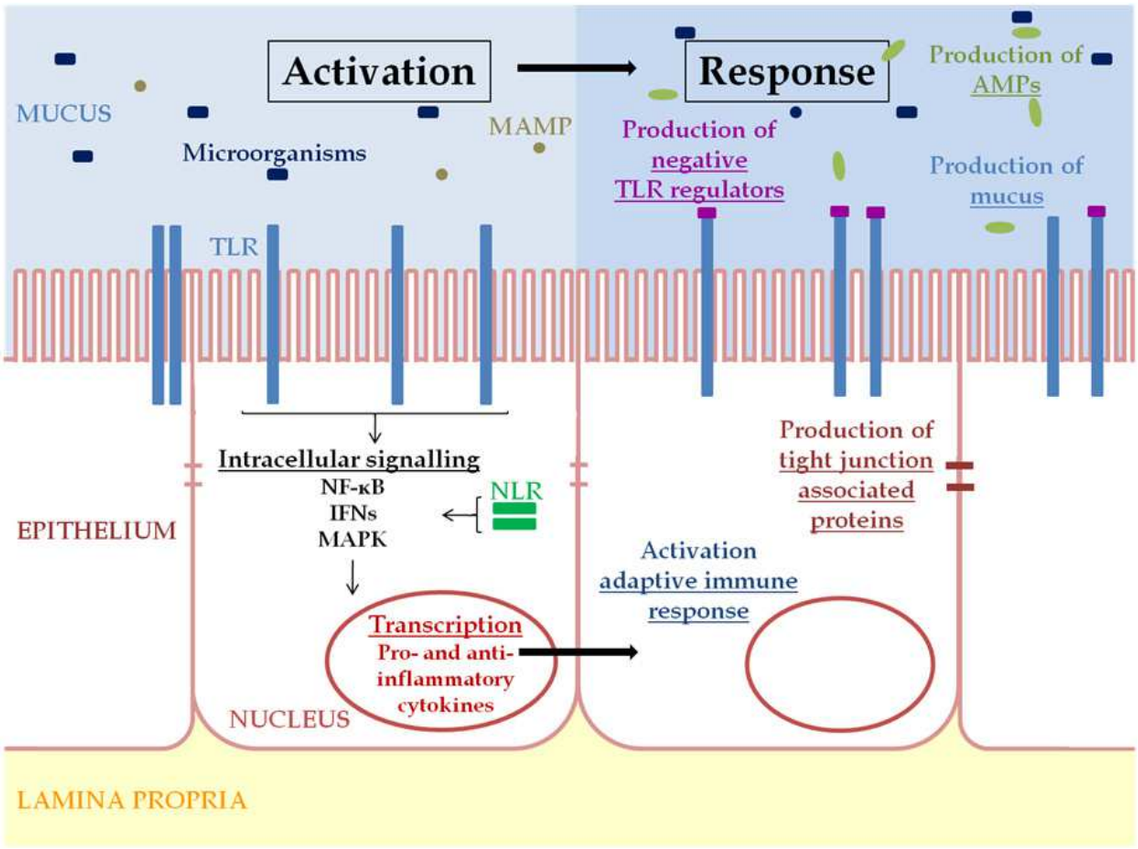

Fig. 4. Schematic overview of the activation and response of the innate immune system. AMP: antimicrobial peptides, IFNs: type I interferons, MAMPs: microbe-associated molecular patterns, MAPK: mitogen-activated protein kinase, NF-κB: nuclear factor-κB, NLR: nucleotide-binding oligomerization domain-like receptors, and TLR: Toll-like receptor

4.2 The role of gut bacteria in inflammation

IBD is a collective term for idiopathic and chronic inflammatory disorders of the intestinal tract. The best known forms of IBD are Crohn's disease (CD) and ulcerative colitis (UC). Research from the last decade has given us new insights into the etiology of IBD although the cause still remains speculative. The most widely accepted theory is that a genetically dysregulated host immune response is over-aggressive against the commensal microbiota (Cho, 2008; Sartor, 2006; Strober et al., 2007).

Genome-wide association (GWA) studies have become a useful and powerful tool to identify disease-associated genes involved in the immunopathogenesis of IBD. The most well-known CD associated gene is NOD2 (also designated CARD15) (Hugot et al., 1996). Three single nucleotide polymorphisms (SNPs) are located within or near the LRR domain of the NOD2 protein, the domain that senses the bacterial products. Studies in primary human cells carrying the 3 major NOD2 SNPs have consistently demonstrated a deficient signalisation pathway what makes an intestinal immune response against the bacteria impossible. In contrast, in mice, NOD2 polymorphisms were shown to potentiate the NF-κB activity (Maeda, 2005; Watanabe et al., 2004). It is clear from these studies that the role of NOD2 mutation in the innate immune signaling is very complex and not completely

understood. Other genes that were associated with CD are two autophagy-related genes ATG16L1 and IRGM (Cho, 2008). GWA studies have further revealed that significant association of numerous SNPs throughout the IL23R gene region are associated with CD and UC (Duerr et al., 2006). Although genetic defects are apparent, they cannot explain completely the increase in the prevalence of IBD.

Several lines of evidence suggest a role for the microbiota in the development of IBD. The most compelling is probably the study by Garrett et al. (2007) in which T-bet deficient mice were used. T-bet regulates the response of the mucosal immune system to the commensal bacteria by controlling TNF-α production. T-bet deficient mice develop a disease that resembles UC. Remarkably, the transmission of the gut microbiota from T-bet deficient mice induced a colitogenic process in T-bet sufficient pups (Garrett et al., 2007). More evidence comes from animal models in which the presence of gut microbiota is required in order to develop intestinal inflammation (Nell et al., 2010; Saleh & Elson, 2011) and antibiotic treatments being effective in subsets of patients with CD (Perencevich & Burakoff, 2006).

One of the theories states that the pathogen *Mycobacterium avium* subspecies paratuberculosis (MAP) is the causative agent for IBD. However, studies based on the detection of MAP by PCR or ELISA in blood samples or biopsies have shown conflicting results (Autschbach et al., 2005; Baksh et al., 2004; Bentley et al., 2008; Clancy et al., 2007; Rowbotham et al., 1995; Wu et al., 1991). More recently, a newly defined *E. coli* pathovar, adherent-invasive *E. coli* (AIEC) was found to be highly associated with ileal mucosa of CD patients (Darfeuille-Michaud et al., 2004).

Other studies focused on a dysbiosis between the commensal microbial communities. Using a wide range of different techniques, changes in the population diversity of both the luminal and mucosa-associated microbiota have been demonstrated. Luminal changes were mainly associated with a decrease in the diversity of the Firmicutes, in particular Lactobacilli and Clostridia, and Bacteroidetes (Manichanh et al., 2006; Marteau, 2009; Scanlan et al., 2006; Sokol et al., 2009), while an increase in the Enterobacteriaceae population was reported for CD (Seksik et al., 2003). All reports described a reduced diversity of the mucosa-associated microbial communities in IBD. The reduction in diversity was comparable to the changes found in the luminal microbiota, i.e. a loss of Firmicutes and Bacteroidetes, particularly Lactobacilli and Eubacterium, with an increase in Enterobacteriaceae species for CD samples (Frank et al., 2007; Nishikawa et al., 2009; Ott et al., 2004; Tamboli et al., 2004). In addition, microscopy studies found an increased bacterial load in the mucosa of IBD patients (Swidsinski et al., 2005). Considering the different studies, no consensus has been reached concerning differences in the diversity of the fecal or mucosa-associated microbiota of IBD patients and healthy volunteers as etiological factor in IBD.

Finally, products of bacterial activity, such as butyrate are also known to have a regulatory effect on inflammation in IBD (Sanderson, 2004; Segain et al., 2000).

4.3 Innovative approaches for drug development in IBD

As dysbiosis in the microbiota is believed to be involved in IBD, strategies to deliberately modulate the microbiota have been developed. These strategies can be of nutritional origin using pro-, pre-, and synbiotics to induce directed changes in the microbial communities leading to health benefits for the host. Probiotics are live microorganisms that confer health benefits when administered in adequate amounts. Prebiotics are indigestible food compounds that selectively stimulate the growth and/or activity of one or a limited number of microbial species in the gut. Synbiotics are a combination of pro- and prebiotics. More

than 25 individual bacterial species (f. ex. *E. coli* Nissle 1917, *Saccharomyces boulardii* 750, *Lactobacillus rahmnosus* LGG), a few formulations containing multiple species (f. ex. VSL#3), short-chain carbohydrates (f. ex. fructooligosaccharides, galactooligosaccharides and arabinoxylans), germinated barley foodstuff or a combination of those have been studied using experimental models of colitis. Evidence from these animal models indicated that probiotics can alter the intestinal microbiota and ameliorate disease (Sartor, 2004) while prebiotic supplementation has been shown to enhance luminal immunoregulatory bacteria, reduce the risk of intestinal infections and the activity of pro-inflammatory transcription factors, and attenuate inflammation and mucosal damage (Cavin et al., 2005; Hedin et al., 2007; Holma et al., 2002; Kanauchi et al., 2008; Komiyama et al., 2011; Sartor, 2004). In addition, prebiotics can stimulate the production of short-chain fatty acids, such as propionate and butyrate, which are believed to improve colonic health. Probiotic therapy with *E. coli* Nissle 1917, bifidobacteria and bifidobacteria fermented milk showed efficacy and safety in maintaining remission of UC and had possible preventive effects on the relapse (Cui et al., 2004; Ishikawa et al., 2003; Kruis et al., 2004). Effectiveness in the induction of remission in UC patients was shown for combined therapy of VSL#3 with balsalazide and direct delivery of *E. coli* Nissle 1917 enemas to the colon (Matthes et al., 2010; Tursi et al., 2004). Germinated barley foodstuff showed a significant decrease in the mean clinical activity of patients with mild- to moderately-active UC (Mitsuyama et al., 1998). Moreover, the combination of oligofructose, inulin, and a probiotic strain (*Bifidobacterium longum*) showed an increase in mucosal-associated bifidobacteria concentrations associated with a decrease in both pro-inflammatory cytokines and antimicrobial defensins. However, the observed alterations in the mucosal cytokine balance was not translated into clinical changes (Furrie et al., 2005). Maintenance of remission in CD patients was reported to be effective when *S. boulardii* combined with the antibiotic mesalazine was administered (Guslandi et al., 2000). However, more studies report that pro-, pre-, and synbiotics are not effective in the induction and maintenance of remission in CD patients (Hedin et al., 2007). To summarize, some human trials indicate the effectiveness of pro-, pre-, and synbiotics in UC but they are scarce in CD. Due to variation in the populations studied, in the distribution of the disease, in the prevalence of genotypes, the small numbers of patients and the lack of details on the diet of the patients (Hedin et al., 2007), there is a marked heterogeneity in the performed studies making a consensus on the treatment of IBD with pro-, pre-, and synbiotics difficult and controversial.

As described above, a strategie which makes actively use of bacterial metabolism to improve drug bioavailability and which is used in the therapy of IBD, is linkage of the drug to a conjugate. Due to the metabolic activity of one or a few species of the microbiota, the release of the active compound is specific for the colon. By using this approach, side effects due to systemic release of the compound can be avoided. The most prevalent example in this category is sulfasalazine, a drug originally designed for the treatment of rheumatoid arthritis that was later discovered to also benefit patients with inflammatory disorders and which is widely used now. Due to the azoreductase activity of the colonic bacteria, the azo-bond connecting the active compound 5-ASA to sulfapyridine, is hydrolyzed. As the azoreductase enzyme is specific for colonic bacteria, 5-ASA is mainly released in the colon (Azad Khan et al., 1982) leading to a highly specific delivery of the active compound. While there is solid data supporting 5-aminosalicylic acid in the induction and maintenance of remission for UC, its efficacy in the treatment of CD is not as clear. One of the aspects involved is the effectiveness of the release of 5-ASA in the small intestines as the colonic release has not occurred yet in this

region. Limitations of sulfasalazine include allergic reactions and side effects, largely attributed to the sulfapyridine moiety. In response, two non-sulfapyridine-containing 5-ASA agents, balsalazide and olsalazine were developed (Patel et al., 2007). In addition, inhibition of sulfide production by 5-aminosalicylic acid-containing drugs was reported and may contribute to their therapeutic effect in UC (Edmond et al., 2003).

Furthermore, bacteria may be used as actual production facilities for local delivery of drugs as described above for carotenoid-producing spore-forming *Bacillus* spp.. A first example for IBD is the strain *Bacillus subtilis* PB6 that has been found to secrete surfactins and cyclic lipopeptides and was investigated for its therapeutic effect. Surfactins have exceptional emulsifying but also antibacterial, antiviral and antitumoral properties. As inhibitors of cytosolic phospholipase A2 (PLA2), surfactins may also function as anti-inflammatory agents. PLA2 is the key enzyme in the production of diverse lipid mediators and is involved in the pathophysiology of IBD. Oral administration of *B. subtilis* PB6 to TNBS-treated rats suppressed the inflammation which was seen on several parameters, i.e. mortality rate, weight gain, colon pathology and weight, and plasma levels of pro- and anti-inflammatory cytokines (Selvam et al., 2009).

The production capacity of a bacterium for a specific compound does not necessarily have to be an intrinsic property of the strain. For example, a specific strain of *Lactococcus lactis* has been genetically modified to secrete human interleukin-10 (IL-10), an anti-inflammatory cytokine. IL-10 is a good candidate for the treatment of IBD, yet direct injection of IL-10 induces several undesired side effects. Local delivery of IL-10 in the colon produced by *Lactococcus lactis* offers great advantages over the standard delivery method as the latter is associated with patient discomfort, various systemic side effects and costly production processes (Steidler et al., 2000). However, the use of genetically modified (GM) organisms in healthcare raises legitimate concerns on deliberate release and potential spread of the GM trait. To prevent spreading of the transgene and the GM bacteria into the environment, the thymidylate synthase gene (*thyA*) was replaced with the expression cassette for *hIL10*. *ThyA* codes for an enzyme necessary for the synthesis of the nucleobase thymine and nucleoside thymidine. As a result, the modified strain can only survive when thymidine or thymine are available in the intestinal environment. When deprived of one of these compounds, thymineless death is rapidly induced, preventing the accumulation of the GM bacteria in the environment. Moreover, as thymineless death results in the fragmentation of the DNA, the chances for uptake of *L. lactis* DNA by other strains are very small (Steidler et al., 2003). Several health authorities and biosafety committees have positively evaluated this containment strategy (Rottiers et al., 2009). A phase I trial with the modified *L. lactis* strain (LL-Thy12) showed promising results as maintenance treatment in CD avoiding systemic side effects (Braat et al., 2006). A second example of a bacterium engineered as production facility is a genetically modified *L. lactis* able to secrete the low calcium response V (LcrV) protein from the enteropathogenic species *Yersinia pseudotuberculosis*. Oral administration of this *L. lactis* induced the expression of IL-10 in the colon and decreased inflammation in 2 murine models of colitis, i.e. TNBS and DSS (Foligne et al., 2007). As these effects were absent in the IL-10$^{-/-}$ mice model, this study once more confirmed the therapeutic potential of IL-10. Recently, Steidler and colleagues proposed *L. lactis* genetically engineered to secrete anti-TNF nanobodies, trefoil factor (TFF) or IL-27 as promising therapeutics for IBD (Durum et al., 2010; Vandenbroucke et al., 2010; Vandenbroucke et al., 2004). These strains have only been tested in preclinical studies.

Table 2 summarizes the different strategies for drug development in which the microbial metabolism plays a key role.

Approach	Species involved	Specific bacterial activity	Advantages	Progress of development	References
Targeted alterations of the gut microbiota	Probiotics f. ex. *E. coli* Nissle 1917, bifidobacteria fermented milk, VSL#3	Modulation of the host-microbe interactions by the growth of health-promoting bacteria	Inhibition of intestinal pathogens Improve epithelial and mucosal barrier function Alteration of the immunoregulation	Commercialized	(Cui et al., 2004; Guslandi et al., 2000; Ishikawa et al., 2003; Kruis et al., 2004; Matthes et al., 2010; Tursi et al., 2004)
	Prebiotics f. ex. germinated barley foodstuff, arabinoxylans			Commercialized	(Komiyama et al., 2011; Mitsuyama et al., 1998)
	Synbiotics f. ex. oligofructose, inulin, and *Bifidobacterium longum*			Commercialized	(Furrie et al., 2005)
Microbial metabolism for colon targeted drug release	Colonic bacteria	Azo-reductase and others for the conversion of the prodrug to the active compound	Specific release of the active compound in the colon	Commercialized f. ex. sulfasalazine, olsalazine, balsalazide	(Azad Khan et al., 1982)
	Colonic bacteria	Azo-reductase and others for the degradation of the polymer capsule		Commercialized f. ex. mesalazine, budesonide	(Chavan et al., 2001; Kimura et al., 1992)
Bacteria as production facilities-	Cyanobacteria, *Burkholderia* spp., Actinomycetes, Myxobacteria	Production of new classes of antibiotics	Natural product to combat resistant pathogens	Preclinical studies	(Donia et al., 2008; Partida-Martinez & Hertweck, 2005; Scott et al., 2008; Wenzel & Muller, 2009)
	Carotenoid producing *Bacillus* spores	Production of carotenoids	Resistant to gastric conditions but germination under colonic conditions Storage at ambient temperature	Preclinical studies	(Duc et al., 2006; Hong et al., 2009; Khaneja et al., 2010; Perez-Fons et al., 2011)
	Bacillus subtilis PB6 spores	Production of surfactins		Preclinical studies	(Selvam et al., 2009)
	Lactococcus lactis	Production of hIL-10	Oral administration Efficient local delivery More favorable side effect profile Cost-efficient manufacturing process	Ongoing large-scale, double-blind, placebo-controlled phase IIA trial	(Braat et al., 2006; Rottiers et al., 2009; Steidler et al., 2000; Steidler et al., 2003)
		Production of Trefoil factor (TFF)		Preclinical studies	(Vandenbroucke et al., 2004)
		Production of low calcium response V (LcrV)		Preclinical studies	(Foligne et al., 2007)
		Production of anti-TNF nanobodies		Preclinical studies	(Vandenbroucke et al., 2010)
		Production of IL-27		Preclinical studies	(Durum et al., 2010)

Table 2. Innovative approaches for the development of biopharmaceuticals making benefit of specific microbial functionalities

5. Conclusion

This chapter has highlighted the enormous potential of the gut microbial metabolism in the modulation of nutritional compounds, drugs and environmental contaminants. We have shown that the intestinal microbiome can be involved in different levels of the ADME characteristics of active compounds. The microbiota can interfere in the absorption of drugs, f. ex. by slow release of the active compound. They can interfere in the metabolism as illustrated by many examples and they may prolong the action of drugs by allowing their enterohepatic circulation to continue and to inhibit excretion. Moreover, the microbial metabolism from ingested compounds can have varying responses, which can be beneficial but can cause some serious risks as well. Due to their interference in ADME and the resulting health effects, a complete understanding of the full metabolic potency of the gut microbiome to predict its modulating effect on xenobiotics is emerging. Gut microbial processes therefore need to be incorporated in pharmacokinetic models.

To achieve full understanding of the microbial potential, the development of suitable *in vitro* models is essential. Two dynamic GIT simulators, the TIM and SHIME® model have been developed for this purpose, each with their own specific advantages an disadvantages to investigate the role of the microbial metabolism on the biotransformation of active compounds. Incorporation of absorptive processes in these models drastically improves the simulation of the bioavailability of the active compounds and by incorporating host cells in the SHIME® model, this model will be a useful tool to evaluate the effect of microbial processes on the host cells and vice versa.

I*n vitro* models do not merely offer opportunities to understand the biotransformation of active compounds but they offer the possibility to investigate the metabolic fate of newly developed drugs. Moreover, new strategies making use of the microbial metabolic potential to improve drug efficacy were discussed going from the local release of active compounds from prodrugs to engineering of strains to secrete specific health promoting compounds. Inflammatory disorders offer the perfect case for the application of these strategies. What has been described until now is only the beginning of a new generation of drugs making use of the enormous potential of the intestinal microbiome.

Finally, given the potential implications the microbiota may have on the stability, bioavailablity and safety of xenobiotics, assessment of the activity of the intestinal microbiome should become a standard process in pharmaceutical drug development. The microbial potential should be further exploited to improve drug development and develop new strategies. By the ongoing technical improvements of *in vitro* models, these offer a valid tool to evaluate the bioavailability of new compounds and their therapeutic effect on host cells. Moreover, as personalized health care is becoming more and more integrated in modern medicine, interindividual variability in the gut microbiome should be an integral part of this process.

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7. References

Agarwal, S., & Rao, A. V. (1998). Tomato Lycopene and Low Density Lipoprotein Oxidation: A Human Dietary Intervention Study. *Lipids,* Vol.33, No.10, (October 1998), pp. 981-984, ISSN 0024-4201

Anson, N. M., Selinheimo, E., Havenaar, R., Aura, A. M., Mattila, I., Lehtinen, P., Bast, A., Poutanen, K., & Haenen, G. (2009a). Bioprocessing of Wheat Bran Improves in Vitro Bioaccessibility and Colonic Metabolism of Phenolic Compounds. *Journal of Agricultural and Food Chemistry,* Vol.57, No.14, (July 2009), pp. 6148-6155, ISSN 0021-8561

Anson, N. M., van den Berg, R., Havenaar, R., Bast, A., & Haenen, G. (2009b). Bioavailability of Ferulic Acid Is Determined by Its Bioaccessibility. *Journal of Cereal Science,* Vol.49, No.2, (March 2009), pp. 296-300, ISSN 0733-5210

Autschbach, F., Eisold, S., Hinz, U., Zinser, S., Linnebacher, M., Giese, T., Loffler, T., Buchler, M. W., & Schmidt, J. (2005). High Prevalence of *Mycobacterium Avium* Subspecies Paratuberculosis IS900 DNA in Gut Tissues from Individuals with Crohn's Disease. *Gut,* Vol.54, No.7, (July 2005), pp. 944-949, ISSN 0017-5749

Azad Khan, A. K., Truelove, S. C., & Aronson, J. K. (1982). The Disposition and Metabolism of Sulphasalazine (Salicylazosulphapyridine) in Man. *British Journal of Pharmacology,* Vol.13, No.4, (April 1982), pp. 523-528, ISSN 0306-5251

Backhed, F., Ley, R. E., Sonnenburg, J. L., Peterson, D. A., & Gordon, J. I. (2005). Host-Bacterial Mutualism in the Human Intestine. *Science,* Vol.307, No.5717, (March 2005), pp. 1915-1920, ISSN 0036-8075

Backhed, F., Manchester, J. K., Semenkovich, C. F., & Gordon, J. I. (2007). Mechanisms Underlying the Resistance to Diet-Induced Obesity in Germ-Free Mice. *Proceedings of the National Academy of Sciences of the United States of America,* Vol.104, No.3, (January 2007), pp. 979-984, ISSN 0027-8424

Baksh, F. K., Finkelstein, S. D., Ariyanayagam-Baksh, S. M., Swalsky, P. A., Klein, E. C., & Dunn, J. C. (2004). Absence of *Mycobacterium avium* Subsp Paratuberculosis in the Microdissected Granulomas of Crohn's Disease. *Modern Pathology,* Vol.17, No.10, (October 2004), pp. 1289-1294, ISSN 0893-3952

Ben-Dor, A., Steiner, M., Gheber, L., Danilenko, M., Dubi, N., Linnewiel, K., Zick, A., Sharoni, Y., & Levy, J. (2005). Carotenoids Activate the Antioxidant Response Element Transcription System. *Molecular Cancer Therapeutics,* Vol.4, No.1, (January 2005), pp. 177-186, ISSN 1535-7163

Bentley, R. W., Keenan, J. I., Gearry, R. B., Kennedy, M. A., Barclay, M. L., & Roberts, R. L. (2008). Incidence of *Mycobacterium avium* Subspecies Paratuberculosis in a Population-Based Cohort of Patients with Crohn's Disease and Control Subjects. *American Journal of Gastroenterology,* Vol.103, No.5, (May 2008), pp. 1168-1172, ISSN 0002-9270

Bertau, M., Brusch, I., & Kummer, U. (2008). Biosimulation of Drug Metabolism, In: *Biosimulation in Drug Development*, Bertau, M., Brusch, I., & Kummer, U. (Eds.), pp.59-85, Wiley-VCH Verlag GmbH & Co. KGaA, ISBN 978-352-7622-67-2, Weinheim, Germany

Blanquet, S., Meunier, J. P., Minekus, M., Marol-Bonnin, S., & Alric, M. (2003). Recombinant *Saccharomyces cerevisiae* Expressing P450 in Artificial Digestive Systems: A Model

for Biodetoxication in the Human Digestive Environment. *Applied and Environmental Microbiology,* Vol.69, No.5, (May 2003), pp. 2884-2892, ISSN 0099-2240

Bolca, S., Possemiers, S., Herregat, A., Huybrechts, I., Heyerick, A., De Vriese, S., Verbruggen, M., Depypere, H., De Keukeleire, D., Bracke, M., De Henauw, S., Verstraete, W., & Van de Wiele, T. (2007a). Microbial and Dietary Factors Are Associated with the Equol Producer Phenotype in Healthy Postmenopausal Women. *Journal of Nutrition,* Vol.137, No.10, (October 2007), pp. 2242-2246, ISSN 0022-3166

Bolca, S., Possemiers, S., Maervoet, V., Huybrechts, I., Heyerick, A., Vervarcke, S., Depypere, H., De Keukeleire, D., Bracke, M., De Henauw, S., Verstraete, W., & Van de Wiele, T. (2007b). Microbial and Dietary Factors Associated with the 8-Prenylnaringenin Producer Phenotype: A Dietary Intervention Trial with Fifty Healthy Post-Menopausal Caucasian Women. *British Journal of Nutrition,* Vol.98, No.5, (November 2007), pp. 950-959, ISSN 0007-1145

Bowes, S. G., & Renwick, A. G. (1986). The Intestinal Metabolism and DNA-Binding of Benzo[a]Pyrene in Guinea-Pigs Fed Normal, High-Fat and High-Cholesterol Diets. *Xenobiotica,* Vol.16, No.6, (June 1986), pp. 543-553, ISSN 0049-8254

Braat, H., Rottiers, P., Hommes, D. W., Huyghebaert, N., Remaut, E., Remon, J. P., Van Deventer, S. J. H., Neirynck, S., Peppelenbosch, M. P., & Steidler, L. (2006). A Phase I Trial with Transgenic Bacteria Expressing Interleukin-10 in Crohn's Disease. *Clinical Gastroenterology and Hepatology,* Vol.4, No.6, (June 2006), pp. 754-759, ISSN 1542-3565

Bugrim, A., Nikolskaya, T., & Nikolsky, Y. (2004). Early Prediction of Drug Metabolism and Toxicity: Systems Biology Approach and Modeling. *Drug Discovery Today,* Vol.9, No.3, (February 2004), pp. 127-135, ISSN 1359-6446

Carroll, M. C. (2004). The Complement System in Regulation of Adaptive Immunity. *Nature Immunology,* Vol.5, No.10, (October 2004), pp. 981-986, ISSN 1529-2908

Cavin, C., Delannoy, M., Malnoe, A., Debefve, E., Touche, A., Courtois, D., & Schilter, B. (2005). Inhibition of the Expression and Activity of Cyclooxygenase-2 by Chicory Extract. *Biochemical and Biophysical Research Communications,* Vol.327, No.3, (February 2005), pp. 742-749, ISSN 0006-291X

Chang, C., & Swaan, P. W. (2006). Computational Approaches to Modeling Drug Transporters. *European Journal of Pharmaceutical Sciences,* Vol.27, No.5, (April 2006), pp. 411-424, ISSN 0928-0987

Chavan, M. S., Sant, V. P., & Nagarsenker, M. S. (2001). Azo-Containing Urethane Analogues for Colonic Drug Delivery: Synthesis, Characterization and in Vitro Evaluation. *Journal of Pharmacy and Pharmacology,* Vol.53, No.6, (June 2001), pp. 895-900, ISSN 0022-3573

Cho, J. H. (2008). The Genetics and Immunopathogenesis of Inflammatory Bowel Disease. *Nature Reviews Immunology,* Vol.8, No.6, (June 2008), pp. 458-466, ISSN 1474-1733

Clancy, R., Ren, Z., Turton, J., Pang, G., & Wettstein, A. (2007). Molecular Evidence for *Mycobacterium avium* Subspecies Paratuberculosis (MAP) in Crohn's Disease Correlates with Enhanced TNF-Alpha Secretion. *Digestive and Liver Disease,* Vol.39, No.5, (May 2007), pp. 445-451, ISSN 1590-8658

Clardy, J., Fischbach, M. A., & Walsh, C. T. (2006). New Antibiotics from Bacterial Natural Products. *Nature Biotechnology,* Vol.24, No.12, (December 2006), pp. 1541-1550, ISSN 1087-0156

Conly, J. M., & Stein, K. (1992). Quantitative and Qualitative Measurements of Vitamin K in Human Intestinal Contents. *American Journal of Gastroenterology,* Vol.87, No.3, (March 1992), pp. 311-316, ISSN 0002-9270

Cooper, M. D., & Alder, M. N. (2006). The Evolution of Adaptive Immune Systems. *Cell,* Vol.124, No.4, (February 2006), pp. 815-822, ISSN 0092-8674

Cui, H. H., Chen, C. L., Wang, J. D., Yang, Y. J., Cun, Y., Wu, J. B., Liu, Y. H., Dan, H. L., Jian, Y. T., & Chen, X. Q. (2004). Effects of Probiotic on Intestinal Mucosa of Patients with Ulcerative Colitis. *World Journal of Gastroenterology,* Vol.10, No.10, (May 2004), pp. 1521-1525, ISSN 1007-9327

Cutting, S. M. (2011). *Bacillus* Probiotics. *Food Microbiology,* Vol.28, No.2, (April 2011), pp. 214-220, ISSN 0740-0020

Darfeuille-Michaud, A., Boudeau, J., Bulois, P., Neut, C., Glasser, A. L., Barnich, N., Bringer, M. A., Swidsinski, A., Beaugerie, L., & Colombel, J. F. (2004). High Prevalence of Adherent-Invasive *Escherichia coli* Associated with Ileal Mucosa in Crohn's Disease. *Gastroenterology,* Vol.127, No.2, (August 2004), pp. 412-421, ISSN 0016-5085

Deat, E., Blanquet-Diot, S., Jarrige, J. F., Denis, S., Beyssac, E., & Alric, M. (2009). Combining the Dynamic TNO-Gastrointestinal Tract System with a Caco-2 Cell Culture Model: Application to the Assessment of Lycopene and Alpha-Tocopherol Bioavailability from a Whole Food. *Journal of Agricultural and Food Chemistry,* Vol.57, No.23, (December 2009), pp. 11314-11320, ISSN 0021-8561

Dethlefsen, L., Eckburg, P. B., Bik, E. M., & Relman, D. A. (2006). Assembly of the Human Intestinal Microbiota. *Trends in Ecology & Evolution,* Vol.21, No.9, (September 2006), pp. 517-523, ISSN 0169-5347

de Witte, P., & Lemli, L. (1990). The Metabolism of Anthranoid Laxatives. *Hepato-Gastroenterology,* Vol.37, No.6, (December 1990), pp. 601-605, ISSN 0172-6390

Diaz-Bone, R. A., & Van de Wiele, T. R. (2009). Biovolatilization of Metal(loid)s by Intestinal Microorganisms in the Simulator of the Human Intestinal Microbial Ecosystem. *Environmental Science & Technology,* Vol.43, No.14, (July 2009), pp. 5249-5256, ISSN 0013-936X

Donia, M. S., Ravel, J., & Schmidt, E. W. (2008). A Global Assembly Line for Cyanobactins. *Nature Chemical Biology,* Vol.4, No.6, (June 2008), pp. 341-343, ISSN 1552-4450

Duc, L. H., Fraser, P. D., Tam, N. K. M., & Cutting, S. M. (2006). Carotenoids Present in Halotolerant *Bacillus* Spore Formers. *Fems Microbiology Letters,* Vol.255, No.2, (February 2006), pp. 215-224, ISSN 0378-1097

Duerr, R. H., Taylor, K. D., Brant, S. R., Rioux, J. D., Silverberg, M. S., Daly, M. J., Steinhart, A. H., Abraham, C., Regueiro, M., Griffiths, A., Dassopoulos, T., Bitton, A., Yang, H. Y., Targan, S., Datta, L. W., Kistner, E. O., Schumm, L. P., Lee, A. T., Gregersen, P. K., Barmada, M. M., Rotter, J. I., Nicolae, D. L., & Cho, J. H. (2006). A Genome-Wide Association Study Identifies IL23R as an Inflammatory Bowel Disease Gene. *Science,* Vol.314, No.5804, (December 2006), pp. 1461-1463, ISSN 0036-8075

Durum, S. K., Li, W. Q., Hixon, J. A., Felber, B., Steidler, L., & Hanson, M. L. (2010). *Lactococcus lactis* Expressing IL-27: Therapeutic Promise in Inflammatory Bowel Disease, *Proceedings of 8th Joint Conference of the International Cytokine Society/International Society for and Cytokine Research*, ISBN 1043-4666, Chicago, IL, USA, October, 2010

Eckburg, P. B., Bik, E. M., Bernstein, C. N., Purdom, E., Dethlefsen, L., Sargent, M., Gill, S. R., Nelson, K. E., & Relman, D. A. (2005). Diversity of the Human Intestinal Microbial Flora. *Science,* Vol.308, No.5728, (May 2005), pp. 1635-1638, ISSN 1095-9203

Edmond, L. M., Hopkins, M. J., Magee, E. A., & Cummings, J. H. (2003). The Effect of 5-Aminosalicylic Acid-Containing Drugs on Sulfide Production by Sulfate-Reducing and Amino Acid-Fermenting Bacteria. *Inflammatory Bowel Diseases,* Vol.9, No.1, (January 2003), pp. 10-17, ISSN 1078-0998

Egert, M., de Graaf, A. A., Smidt, H., de Vos, W. M., & Venema, K. (2006). Beyond Diversity: Functional Microbiomics of the Human Colon. *Trends in Microbiology,* Vol.14, No.2, (February 2006), pp. 86-91, ISSN 0966-842X

Elson, C. O., Cong, Y., Qi, F., Hershberg, R. M., & Targan, S. R. (2006). Molecular Approaches to the Role of the Microbiota in Iflammatory Bowel Disease. *Proceedings of the International Symposium on Inflammatory Bowel Diseases,* ISBN 0077-8923, Munster, Germany, September 2005

Fischbach, M. A., & Walsh, C. T. (2009). Antibiotics for Emerging Pathogens. *Science,* Vol.325, No.5944, (August 2009), pp. 1089-1093, ISSN 0036-8075

Foligne, B., Dessein, R., Marceau, M., Poiret, S., Chamaillard, M., Pot, B., Simonet, M., & Daniel, C. (2007). Prevention and Treatment of Colitis with *Lactococcus lactis* Secreting the Immunomodulatory *Yersinia* LcrV Protein. *Gastroenterology,* Vol.133, No.3, (September 2007), pp. 862-874, ISSN 0016-5085

Frank, D. N., Amand, A. L. S., Feldman, R. A., Boedeker, E. C., Harpaz, N., & Pace, N. R. (2007). Molecular-Phylogenetic Characterization of Microbial Community Imbalances in Human Inflammatory Bowel Diseases. *Proceedings of the National Academy of Sciences of the United States of America,* Vol.104, No.34, (August 2007), pp. 13780-13785, ISSN 0027-8424

Fuhrman, B., Elis, A., & Aviram, M. (1997). Hypocholesterolemic Effect of Lycopene and Beta-Carotene Is Related to Suppression of Cholesterol Synthesis and Augmentation of LDL Receptor Activity in Macrophages. *Biochemical and Biophysical Research Communications,* Vol.233, No.3, (April 1997), pp. 658-662, ISSN 0006-291X

Furrie, E., Macfarlane, S., Kennedy, A., Cummings, J. H., Walsh, S. V., O'Neil, D. A., & Macfarlane, G. T. (2005). Synbiotic Therapy (*Bifidobacterium longum*/Synergy 1) Initiates Resolution of Inflammation in Patients with Active Ulcerative Colitis: A Randomised Controlled Pilot Trial. *Gut,* Vol.54, No.2, (February 2005), pp. 242-249, ISSN 0017-5749

Garrett, W. S., Lord, G. M., Punit, S., Lugo-Villarino, G., Mazmanian, S. K., Ito, S., Glickman, J. N., & Glimcher, L. H. (2007). Communicable Ulcerative Colitis Induced by T-Bet Deficiency in the Innate Immune System. *Cell,* Vol.131, No.1, (October 2007), pp. 33-45, ISSN 0092-8674

Gaskins, H. R. (1997). Immunological Aspects of Host/Microbiota Interactions at the Intestinal Epithelium, In: *Gastrointestinal Microbiology Vol. 2*, Mackie, R. I., White, B. A., & Isaacson, R. E. (Eds.), pp. 537-587, Chapman and Hall, ISBN 041-298-3710, New york, USA

Giovannucci, E. (2002). A Review of Epidemiologic Studies of Tomatoes, Lycopene, and Prostate Cancer. *Experimental Biology and Medicine,* Vol.227, No.10, (November 2002), pp. 852-859, ISSN 1535-3702

Grootaert, C., Van den Abbeele, P., Marzorati, M., Broekaert, W. F., Courtin, C. M., Delcour, J. A., Verstraete, W., & Van de Wiele, T. (2009). Comparison of Prebiotic Effects of Arabinoxylan Oligosaccharides and Inulin in a Simulator of the Human Intestinal Microbial Ecosystem. *FEMS Microbiology Ecology,* Vol.69, No.2, (August 2009), pp. 231-242, ISSN 0168-6496

Guslandi, M., Mezzi, G., Sorghi, M., & Testoni, P. A. (2000). *Saccharomyces boulardii* in Maintenance Treatment of Crohn's Disease. *Digestive Diseases and Sciences,* Vol.45, No.7, (July 2000), pp. 1462-1464, ISSN 0163-2116

Hedin, C., Whelani, K., & Lindsay, J. O. (2007). Evidence for the Use of Probiotics and Prebiotics in Inflammatory Bowel Disease: A Review of Clinical Trials. *Proceedings of the Nutrition Society,* Vol.66, No.3, (August 2007), pp. 307-315, ISSN 0029-6651

Holma, R., Juvonen, P., Asmawi, M. Z., Vapaatalo, H., & Korpela, R. (2002). Galacto-Oligosaccharides Stimulate the Growth of Bifidobacteria but Fail to Attenuate Inflammation in Experimental Colitis in Rats. *Scandinavian Journal of Gastroenterology,* Vol.37, No.9, (September 2002), pp. 1042-1047, ISSN 0036-5521

Hong, H. A., Khaneja, R., Tam, N. M. K., Cazzato, A., Tan, S., Urdaci, M., Brisson, A., Gasbarrini, A., Barnes, I., & Cutting, S. M. (2009). *Bacillus subtilis* Isolated from the Human Gastrointestinal Tract. *Research in Microbiology,* Vol.160, No.2, (March 2009), pp. 134-143, ISSN 0923-2508

Hopkins, M. J., & Macfarlane, G. T. (2003). Nondigestible Oligosaccharides Enhance Bacterial Colonization Resistance against *Clostridium difficile* in Vitro. *Applied and Environmental Microbiology,* Vol.69, No.4, (April 2003), pp. 1920-1927, ISSN 0099-2240

Hugot, J. P., Laurent-Puig, P., Gower-Rousseau, C., Olson, J. M., Lee, J. C., Beaugerie, L., Naom, I., Dupas, J. L., Van Gossum, A., Orholm, M., Bonaiti-Pellie, C., Weissenbach, J., Mathew, C. G., Lennard-Jones, J. E., Cortot, A., Colombel, J. F., & Thomas, G. (1996). Mapping of a Susceptibility Locus for Crohn's Disease on Chromosome 16. *Nature,* Vol.379, No.6568, (February 1996), pp. 821-823, ISSN 0028-0836

Humblot, C., Combourieu, B., Vaisanen, M. L., Furet, J. P., Delort, A. M., & Rabot, S. (2005). 1H Nuclear Magnetic Resonance Spectroscopy-Based Studies of the Metabolism of Food-Borne Carcinogen 2-Amino-3-Methylimidazo[4,5-F]Quinoline by Human Intestinal Microbiota. *Applied and Environmental Microbiology,* Vol.71, No.9, (September 2005), pp. 5116-5123, ISSN 0099-2240

Humblot, C., Murkovic, M., Rigottier-Gois, L., Bensaada, M., Bouclet, A., Andrieux, C., Anba, J., & Rabot, S. (2007). Beta-Glucuronidase in Human Intestinal Microbiota Is Necessary for the Colonic Genotoxicity of the Food-Borne Carcinogen 2-Amino-3-Methylimidazo[4,5-F]Quinoline in Rats. *Carcinogenesis,* Vol.28, No.11, (November 2007), pp. 2419-2425, ISSN 0143-3334

Ilett, K. F., Tee, L. B. G., Reeves, P. T., & Minchin, R. F. (1990). Metabolism of Drugs and Other Xenobiotics in the Gut Lumen and Wall. *Pharmacology & Therapeutics,* Vol.46, No.1, (1990), pp. 67-93, ISSN 0163-7258

Ishikawa, H., Akedo, I., Umesaki, Y., Tanaka, R., Imaoka, A., & Otani, T. (2003). Randomized Controlled Trial of the Effect of Bifidobacteria-Fermented Milk on Ulcerative Colitis. *Journal of the American College of Nutrition,* Vol.22, No.1, (February 2003), pp. 56-63, ISSN 0731-5724

Johansson, M. E. V., Phillipson, M., Petersson, J., Velcich, A., Holm, L., & Hansson, G. C. (2008). The Inner of the Two Muc2 Mucin-Dependent Mucus Layers in Colon Is Devoid of Bacteria. *Proceedings of the National Academy of Sciences of the United States of America,* Vol.105, No.39, (September 2008), pp. 15064-15069, ISSN 0027-8424

Kanauchi, O., Oshima, T., Andoh, A., Shioya, M., & Mitsuyama, K. (2008). Germinated Barley Foodstuff Ameliorates Inflammation in Mice with Colitis through Modulation of Mucosal Immune System. *Scandinavian Journal of Gastroenterology,* Vol.43, No.11, (January 2008), pp. 1346-1352, ISSN 0036-5521

Kawai, T., & Akira, S. (2010). The Role of Pattern-Recognition Receptors in Innate Immunity: Update on Toll-Like Receptors. *Nature Immunology,* Vol.11, No.5, (May 2010), pp. 373-384, ISSN 1529-2908

Kelly, D., Conway, S., & Aminov, R. (2005). Commensal Gut Bacteria: Mechanisms of Immune Modulation. *Trends in Immunology,* Vol.26, No.6, (June 2005), pp. 326-333, ISSN 1471-4906

Khaneja, R., Perez-Fons, L., Fakhry, S., Baccigalupi, L., Steiger, S., To, E., Sandmann, G., Dong, T. C., Ricca, E., Fraser, P. D., & Cutting, S. M. (2010). Carotenoids Found in *Bacillus*. *Journal of Applied Microbiology,* Vol.108, No.6, (June 2010), pp. 1889-1902, ISSN 1364-5072

Kimura, Y., Makita, Y., Kumagai, T., Yamane, H., Kitao, T., Sasatani, H., & Kim, S. I. (1992). Degradation of Azo-Containing Polyurethane by the Action of Intestinal Flora - Its Mechanism and Application as a Drug Delivery System. *Polymer,* Vol.33, No.24, (1992), pp. 5294-5299, ISSN 0032-3861

Komiyama, Y., Andoh, A., Fujiwara, D., Ohmae, H., Araki, Y., Fujiyama, Y., Mitsuyama, K., & Kanauchi, O. (2011). New Prebiotics from Rice Bran Ameliorate Inflammation in Murine Colitis Models through the Modulation of Intestinal Homeostasis and the Mucosal Immune System. *Scandinavian Journal of Gastroenterology,* Vol.46, No.1, (January 2011), pp. 40-52, ISSN 0036-5521

Kruis, W., Fric, P., Pokrotnieks, J., Lukas, M., Fixa, B., Kascak, M., Kamm, M. A., Weismueller, J., Beglinger, C., Stolte, M., Wolff, C., & Schulze, J. (2004). Maintaining Remission of Ulcerative Colitis with the Probiotic *Escherichia coli* Nissle 1917 Is as Effective as with Standard Mesalazine. *Gut,* Vol.53, No.11, (November 2004), pp. 1617-1623, ISSN 0017-5749

Leahy, D. E. (2003). Progress in Simulation Modelling for Pharmacokinetics. *Current Topics in Medicinal Chemistry,* Vol.3, No.11, (July 2003), pp. 1257-1268, ISSN 1568-0266

Ley, R. E., Hamady, M., Lozupone, C., Turnbaugh, P. J., Ramey, R. R., Bircher, J. S., Schlegel, M. L., Tucker, T. A., Schrenzel, M. D., Knight, R., & Gordon, J. I. (2008). Evolution of Mammals and Their Gut Microbes. *Science,* Vol.320, No.5883, (June 2008), pp. 1647-1651, ISSN 0036-8075

Ley, R. E., Peterson, D. A., & Gordon, J. I. (2006). Ecological and Evolutionary Forces Shaping Microbial Diversity in the Human Intestine. *Cell,* Vol.124, No.4, (February 2006), pp. 837-848, ISSN 0092-8674

MacDonald, T. T., & Monteleone, G. (2005). Immunity, Inflammation, and Allergy in the Gut. *Science,* Vol.307, No.5717, (March 2005), pp. 1920-1925, ISSN 0036-8075

Macfarlane, S., & Dillon, J. F. (2007). Microbial Biofilms in the Human Gastrointestinal Tract. *Journal of Applied Microbiology,* Vol.102, No.5, (May 2007), pp. 1187-1196, ISSN 1364-5072

Maeda, S., Hsu, L. C., Liu, H., Bankston, L. A., Iimura, M., Kagnoff, M. F., Eckmann, L., & Karin, M. (2005). NOD2 Mutation in Crohn's Disease Potentiates NF-Kappa B Activity and IL-1 Beta Processing. *Science,* Vol.307, No.5710, (April 2005), pp. 734-738, 0036-8075

Manichanh, C., Rigottier-Gois, L., Bonnaud, E., Gloux, K., Pelletier, E., Frangeul, L., Nalin, R., Jarrin, C., Chardon, P., Marteau, P., Roca, J., & Dore, J. (2006). Reduced Diversity of Faecal Microbiota in Crohn's Disease Revealed by a Metagenomic Approach. *Gut,* Vol.55, No.2, (February 2006), pp. 205-211, ISSN 0017-5749

Mares-Perlman, J. A., Millen, A. E., Ficek, T. L., & Hankinson, S. E. (2002). The Body of Evidence to Support a Protective Role for Lutein and Zeaxanthin in Delaying Chronic Disease. Overview. *Journal of Nutrition,* Vol.132, No.3, (March 2002), pp. 518S-524S, ISSN 0022-3166

Marteau, P. (2009). Bacterial Flora in Inflammatory Bowel Disease. *Digestive Diseases,* Vol.27, (March 2009), pp. 99-103, ISSN 0257-2753

Marzorati, M., Possemiers, S., Van den Abbeele, P., Van de Wiele, T., Vanhoecke, B., & Verstraete, W. (2010). Technology and Method to Study Microbial Growth and Adhesion to Host-Related Surface and the Host-Microbiota Interactions. WO Patent 2010/118857 A2, filed April 14, 2010

Marzorati, M., Possemiers, S., & Verstraete, W. (2009). The Use of the SHIME-Related Technology Platform to Assess the Efficacy of Pre- and Probiotics. *Agro Food Industry Hi-Tech,* Vol.20, No.3, (May-June 2009), pp. 50-53, ISSN 1722-6996

Marzorati, M., Vanhoecke, B., De Ryck, T., Possemiers, S., Van den Abbeele, P., Derycke, L., Bracke, M., Pieters, J., Verstraete, W., & Van de Wiele, T. (2011). Hmi Module: A New Tool to Study in Vitro the Host-Microbiota Interaction in the Gastrointestinal Tract. "submitted for publication"

Matthes, H., Krummenerl, T., Giensch, M., Wolff, C., & Schulze, J. (2010). Clinical Trial: Probiotic Treatment of Acute Distal Ulcerative Colitis with Rectally Administered *Escherichia coli* Nissle 1917 (EcN). *BMC Complementary and Alternative Medicine,* Vol.10, (April 2010), pp. 8, ISSN 1472-6882

Medzhitov, R. (2007). Recognition of Microorganisms and Activation of the Immune Response. *Nature,* Vol.449, No.7164, (October 2007), pp. 819-826, ISSN 0028-0836

Meyer, J., Michalke, K., Kouril, T., & Hensel, R. (2008). Volatilisation of Metals and Metalloids: An Inherent Feature of Methanoarchaea? *Systematic and Applied Microbiology,* Vol.31, No.2, (June 2008), pp. 81-87, ISSN 0723-2020

Michalke, K., Schmidt, A., Huber, B., Meyer, J., Sulkowski, M., Hirner, A. V., Boertz, J., Mosel, F., Dammann, P., Hilken, G., Hedrich, H. J., Dorsch, M., Rettenmeier, A. W., & Hensel, R. (2008). Role of Intestinal Microbiota in Transformation of Bismuth and Other Metals and Metalloids into Volatile Methyl and Hydride Derivatives in Humans and Mice. *Applied and Environmental Microbiology,* Vol.74, No.10, (May 2008), pp. 3069-3075, ISSN 0099-2240

Minekus, M., & Havenaar, R. (1996). In Vitro Model of an in Vivo Digestive Tract. U.S. Patent 5,525,305, filed November 1, 1993, and issued June 11, 1996

Minekus, M., Smeets-Peeters, M., Bernalier, A., Marol-Bonnin, S., Havenaar, R., Marteau, P., Alric, M., Fonty, G., & Veld, J. (1999). A Computer-Controlled System to Simulate Conditions of the Large Intestine with Peristaltic Mixing, Water Absorption and Absorption of Fermentation Products. *Applied Microbiology and Biotechnology,* Vol.53, No.1, (December 1999), pp. 108-114, ISSN 0175-7598

Mitsuyama, K., Saiki, T., Kanauchi, O., Iwanaga, T., Tomiyasu, N., Nishiyama, T., Tateishi, H., Shirachi, A., Ide, M., Suzuki, A., Noguchi, K., Ikeda, H., Toyonaga, A., & Sata, M. (1998). Treatment of Ulcerative Colitis with Germinated Barley Foodstuff Feeding: A Pilot Study. *Alimentary Pharmacology & Therapeutics,* Vol.12, No.12, (December 1998), pp. 1225-1230, ISSN 0269-2813

Molly, K., Woestyne, M. V., & Verstraete, W. (1993). Development of a 5-Step Multichamber Reactor as a Simulation of the Human Intestinal Microbial Ecosystem. *Applied Microbiology and Biotechnology,* Vol.39, No.2, (May 1993), pp. 254-258, ISSN 0175-7598

Nell, S., Suerbaum, S., & Josenhans, C. (2010). The Impact of the Microbiota on the Pathogenesis of IBD: Lessons from Mouse Infection Models. *Nature Reviews Microbiology,* Vol.8, No.8, (Augustus 2010), pp. 564-577, ISSN 1740-1526

Nishikawa, J., Kudo, T., Sakata, S., Benno, Y., & Sugiyama, T. (2009). Diversity of Mucosa-Associated Microbiota in Active and Inactive Ulcerative Colitis. *Scandinavian Journal of Gastroenterology,* Vol.44, No.2, (January 2009), pp. 180-186, ISSN 0036-5521

Nusko, G., Schneider, B., Schneider, I., Wittekind, C., & Hahn, E. G. (2000) Anthranoid Laxative Use is Not a Risk Factor for Colorectal Neoplasia: Results of a Prospective Case Control Study. *Gut,* Vol.46, No.5, (May 2000), pp. 651-655, ISSN 0017-5749

O'Malley, C. D., & Saunders, J. B. de C. M. (Eds.). (1982). *Leonardo Da Vinci on the Human Body: The Anatomical, Physiological, and Embryological Drawings of Leonardo Da Vinci*, Crown Publishers, ISBN 9780-5173-810-52, New York, USA

Okuda, H., Ogura, K., Kato, A., Takubo, H., & Watabe, T. (1998). A Possible Mechanism of Eighteen Patient Deaths Caused by Interactions of Sorivudine, a New Antiviral Drug, with Oral 5-Fluorouracil Prodrugs. *Journal of Pharmacology and Experimental Therapeutics,* Vol.287, No.2, (November 1998), pp. 791-799, ISSN 0022-3565

Ott, S. J., Musfeldt, M., Wenderoth, D. F., Hampe, J., Brant, O., Folsch, U. R., Timmins, K. N., & Schreiber, S. (2004). Reduction in Diversity of the Colonic Mucosa Associated Bacterial Microflora in Patients with Active Inflammatory Bowel Disease. *Gut,* Vol.53, No.5, (May 2004), pp. 685-693, ISSN 0017-5749

Partida-Martinez, L. P., & Hertweck, C. (2005). Pathogenic Fungus Harbours Endosymbiotic Bacteria for Toxin Production. *Nature,* Vol.437, No.7060, (October 2005), pp. 884-888, ISSN 0028-0836

Patel, M., Shah, T., & Amin, A. (2007). Therapeutic Opportunities in Colon-Specific Drug-Delivery Systems. *Critical Reviews in Therapeutic Drug Carrier Systems,* Vol.24, No.2, (March 2007), pp. 147-202, ISSN 0743-4863

Perencevich, M., & Burakoff, R. (2006). Use of Antibiotics in the Treatment of Inflammatory Bowel Disease. *Inflammatory Bowel Diseases,* Vol.12, No.7, (July 2006), pp. 651-664, ISSN 1078-0998

Perez-Fons, L., Steiger, S., Khaneja, R., Bramley, P. M., Cutting, S. M., Sandmann, G., & Fraser, P. D. (2011). Identification and the Developmental Formation of Carotenoid Pigments in the Yellow/Orange *Bacillus* Spore-Formers. *Biochimica Et Biophysica Acta-Molecular and Cell Biology of Lipids,* Vol.1811, No.3, (March 2011), pp. 177-185, ISSN 1388-1981

Pieper, I. A., & Bertau, M. (2010). Predictive Tools for the Evaluation of Microbial Effects on Drugs During Gastrointestinal Passage. *Expert Opinion on Drug Metabolism & Toxicology,* Vol.6, No.6, (June 2010), pp. 747-760, ISSN 1742-5255

Pinyayev, T. S., Kohan, M. J., Herbin-Davis, K., Creed, J. T., & Thomas, D. J. (2011). Preabsorptive Metabolism of Sodium Arsenate by Anaerobic Microbiota of Mouse Cecum Forms a Variety of Methylated and Thiolated Arsenicals. *Chemical Research in Toxicology,* Vol.24, No.4, (April 2011), pp. 475-477, ISSN 0893-228X

Possemiers, S., Bolca, S., Grootaert, C., Heyerick, A., Decroos, K., Dhooge, W., De Keukeleire, D., Rabot, S., Verstraete, W., & Van de Wiele, T. (2006). The Prenylflavonoid Isoxanthohumol from Hops (*Humulus Lupulus* L.) Is Activated into the Potent Phytoestrogen 8-Prenylnaringenin in Vitro and in the Human Intestine. *Journal of Nutrition,* Vol.136, No.7, (July 2006), pp. 1862-1867, ISSN 0022-3166

Possemiers, S., Bolca, S., Verstraete, W., & Heyerick, A. (2011a). The Intestinal Microbiome: A Separate Organ inside the Body with the Metabolic Potential to Influence the Bioactivity of Botanicals. *Fitoterapia,* Vol.82, No.1, (January 2011), pp. 53-66, ISSN 0367-326X

Possemiers, S., Grootaert, C., Vermeiren, J., Gross, G., Marzorati, M., Verstraete, W., & Van de Wiele, T. (2009). The Intestinal Environment in Health and Disease - Recent Insights on the Potential of Intestinal Bacteria to Influence Human Health. *Current Pharmaceutical Design,* Vol.15, No.18, (June 2009), pp. 2051-2065, ISSN 1381-6128

Possemiers, S., Rabot, S., Espin, J. C., Bruneau, A., Philippe, C., Gonzalez-Sarrias, A., Heyerick, A., Tomas-Barberan, F. A., De Keukeleire, D., & Verstraete, W. (2008). *Eubacterium limosum* Activates Isoxanthohumol from Hops (*Humulus Lupulus* L.) into the Potent Phytoestrogen 8-Prenylnaringenin in Vitro and in Rat Intestine. *Journal of Nutrition,* Vol.138, No.7, (July 2008), pp. 1310-1316, ISSN 0022-3166

Possemiers, S., Verhelst, A., Maignien, L., Van den Abbeele, P., Reeves, S. G., Robinson, L. E., Van de Wiele, T., & Marzorati, M. (2011b). A Yeast-Based Fermentate Selectively Modulates Both the Luminal and Mucosal Gut Microbiota, Enhances Butyrate Production and Protects against Inflammation, as Studied in an Integrated in Vitro Approach. "submitted for publication"

Possemiers, S., Verthe, K., Uyttendaele, S., & Verstraete, W. (2004). PCR-DGGE-Based Quantification of Stability of the Microbial Community in a Simulator of the Human Intestinal Microbial Ecosystem. *FEMS Microbiology Ecology,* Vol.49, No.3, (May 2004), pp. 495-507, ISSN 0168-6496

Rottiers, P., De Smedt, T., & Steidler, L. (2009). Modulation of Gut-Associated Lymphoid Tissue Functions with Genetically Modified *Lactococcus lactis*. *International Reviews of Immunology,* Vol.28, No.6, (December 2009), pp. 465-486, ISSN 0883-0185

Rowbotham, D. S., Mapstone, N. P., Trejdosiewicz, L. K., Howdle, P. D., & Quirke, P. (1995). *Mycobacterium*-Paratuberculosis DNA Not Detected in Crohn's Disease Tissue by Fluorescent Polymerase Chain-Reaction. *Gut,* Vol.37, No.5, (November 1995), pp. 660-667, ISSN 0017-5749

Rowland, I., Faughnan, M., Hoey, L., Wahala, K., Williamson, G., & Cassidy, A. (2003). Bioavailability of Phyto-Oestrogens. *British Journal of Nutrition,* Vol.89, (June 2003), pp. S45-S58, ISSN 0007-1145

Saleh, M., & Elson, C. O. (2011). Experimental Inflammatory Bowel Disease: Insights into the Host-Microbiota Dialog. *Immunity,* Vol.34, No.3, (March 2011), pp. 293-302, ISSN 1074-7613

Salminen, S., Bouley, C., Boutron-Ruault, M. C., Cummings, J. H., Franck, A., Gibson, G. R., Isolauri, E., Moreau, M. C., Roberfroid, M., & Rowland, I. (1998). Functional Food

Science and Gastrointestinal Physiology and Function. *British Journal of Nutrition,* Vol.80, (August 1998), pp. S147-S171, ISSN 0007-1145

Salzman, N. H., Hung, K. C., Haribhai, D., Chu, H. T., Karlsson-Sjoberg, J., Amir, E., Teggatz, P., Barman, M., Hayward, M., Eastwood, D., Stoel, M., Zhou, Y. J., Sodergren, E., Weinstock, G. M., Bevins, C. L., Williams, C. B., & Bos, N. A. (2010). Enteric Defensins Are Essential Regulators of Intestinal Microbial Ecology. *Nature Immunology,* Vol.11, No.1, (January 2010), pp. 76-U71, ISSN 1529-2908

Sanderson, I. R. (2004). Short Chain Fatty Acid Regulation of Signaling Genes Expressed by the Intestinal Epithelium. *Journal of Nutrition,* Vol.134, No.9, (September 2004), pp. 2450S-2454S, ISSN 0022-3166

Sanderson, I. R., & Walker, W. A. (2007). Tlrs in the Gut I. The Role of TLRs/NODs in Intestinal Development and Homeostasis. *American Journal of Physiology-Gastrointestinal and Liver Physiology,* Vol.292, No.1, (January 2007), pp. G6-G10, ISSN 0193-1857

Sartor, R. B. (2004). Therapeutic Manipulation of the Enteric Microflora in Inflammatory Bowel Diseases: Antibiotics, Probiotics, and Prebiotics. *Gastroenterology,* Vol.126, No.6, (May 2004), pp. 1620-1633, ISSN 0016-5085

Sartor, R. B. (2006). Mechanisms of Disease: Pathogenesis of Crohn's Disease and Ulcerative Colitis. *Nature Clinical Practice Gastroenterology & Hepatology,* Vol.3, No.7, (July 2006), pp. 390-407, ISSN 1743-4378

Scalbert, A., Manach, C., Morand, C., Remesy, C., & Jimenez, L. (2005). Dietary Polyphenols and the Prevention of Diseases. *Critical Reviews in Food Science and Nutrition,* Vol.45, No.4, (June 2005), pp. 287-306, ISSN 1040-8398

Scanlan, P. D., Shanahan, F., O'Mahony, C., & Marchesi, J. R. (2006). Culture-Independent Analyses of Temporal Variation of the Dominant Fecal Microbiota and Targeted Bacterial Subgroups in Crohn's Disease. *Journal of Clinical Microbiology,* Vol.44, No.11, (November 2006), pp. 3980-3988, ISSN 0095-1137

Scott, J. J., Oh, D. C., Yuceer, M. C., Klepzig, K. D., Clardy, J., & Currie, C. R. (2008). Bacterial Protection of Beetle-Fungus Mutualism. *Science,* Vol.322, No.5898, (October 2008), pp. 63-63, ISSN 0036-8075

Segain, J. P., de la Bletiere, D. R., Bourreille, A., Leray, V., Gervois, N., Rosales, C., Ferrier, L., Bonnet, C., Blottiere, H. M., & Galmiche, J. P. (2000). Butyrate Inhibits Inflammatory Responses through NF Kappa B Inhibition: Implications for Crohn's Disease. *Gut,* Vol.47, No.3, (September 2000), pp. 397-403, ISSN 0017-5749

Seksik, P., Rigottier-Gois, L., Gramet, G., Sutren, M., Pochart, P., Marteau, P., Jian, R., & Dore, J. (2003). Alterations of the Dominant Faecal Bacterial Groups in Patients with Crohn's Disease of the Colon. *Gut,* Vol.52, No.2, (February 2003), pp. 237-242, ISSN 0017-5749

Selvam, R., Maheswari, P., Kavitha, P., Ravichandra, M., Sas, B., & Ramchand, C. N. (2009). Effect of *Bacillus subtilis* PB6, a Natural Probiotic on Colon Mucosal Inflammation and Plasma Cytokines Levels in Inflammatory Bowel Disease. *Indian Journal of Biochemistry & Biophysics,* Vol.46, No.1, (February 2009), pp. 79-85, ISSN 0301-1208

Smit, P., & Heniger, J. (1975). Antoni Van Leeuwenhoek (1632-1723) and the Discovery of Bacteria. *Antonie Van Leeuwenhoek Journal of Microbiology,* Vol.41, No.3, (1975), pp. 217-228, ISSN 0003-6072

Sokol, H., Seksik, P., Furet, J. P., Firmesse, O., Nion-Larmurier, L., Beaugerie, L., Cosnes, J., Corthier, G., Marteau, P., & Dore, J. (2009). Low Counts of *Faecalibacterium*

prausnitzii in Colitis Microbiota. *Inflammatory Bowel Diseases,* Vol.15, No.8, (August 2009), pp. 1183-1189, ISSN 1078-0998

Sousa, T., Paterson, R., Moore, V., Carlsson, A., Abrahamsson, B., & Basit, A. W. (2008). The Gastrointestinal Microbiota as a Site for the Biotransformation of Drugs. *International Journal of Pharmaceutics,* Vol.363, No.1-2, (November 2008), pp. 1-25, ISSN 0378-5173

Steidler, L., Hans, W., Schotte, L., Neirynck, S., Obermeier, F., Falk, W., Fiers, W., & Remaut, E. (2000). Treatment of Murine Colitis by *Lactococcus lactis* Secreting Interleukin-10. *Science,* Vol.289, No.5483, (August 2000), pp. 1352-1355, ISSN 0036-8075

Steidler, L., Neirynck, S., Huyghebaert, N., Snoeck, V., Vermeire, A., Goddeeris, B., Cox, E., Remon, J. P., & Remaut, E. (2003). Biological Containment of Genetically Modified *Lactococcus lactis* for Intestinal Delivery of Human Interleukin 10. *Nature Biotechnology,* Vol.21, No.7, (July 2003), pp. 785-789, ISSN 1087-0156

Strober, W., Fuss, I., & Mannon, P. (2007). The Fundamental Basis of Inflammatory Bowel Disease. *Journal of Clinical Investigation,* Vol.117, No.3, (March 2007), pp. 514-521, ISSN 0021-9738

Su, L. P., Shen, L., Clayburgh, D. R., Nalle, S. C., Sullivan, E. A., Meddings, J. B., Abraham, C., & Turner, J. R. (2009). Targeted Epithelial Tight Junction Dysfunction Causes Immune Activation and Contributes to Development of Experimental Colitis. *Gastroenterology,* Vol.136, No.2, (February 2009), pp. 551-563, ISSN 0016-5085

Sugano, K. (2009). Introduction to Computational Oral Absorption Simulation. *Expert Opinion on Drug Metabolism & Toxicology,* Vol.5, No.3, (March 2009), pp. 259-293, ISSN 1742-5255

Swidsinski, A., Weber, J., Loening-Baucke, V., Hale, L. P., & Lochs, H. (2005). Spatial Organization and Composition of the Mucosal Flora in Patients with Inflammatory Bowel Disease. *Journal of Clinical Microbiology,* Vol.43, No.7, (July 2005), pp. 3380-3389, ISSN 0095-1137

Tamboli, C. P., Neut, C., Desreumaux, P., & Colombel, J. F. (2004). Dysbiosis in Inflammatory Bowel Disease. *Gut,* Vol.53, No.1, (January 2004), pp. 1-4, ISSN 0017-5749

Turnbaugh, P. J., Hamady, M., Yatsunenko, T., Cantarel, B. L., Duncan, A., Ley, R. E., Sogin, M. L., Jones, W. J., Roe, B. A., Affourtit, J. P., Egholm, M., Henrissat, B., Heath, A. C., Knight, R., & Gordon, J. I. (2009). A Core Gut Microbiome in Obese and Lean Twins. *Nature,* Vol.457, No.7228, (January 2009), pp. 480-484, ISSN 0028-0836

Tursi, A., Brandimarte, G., Giorgetti, G. M., Forti, G., Modeo, M. E., & Gigliobianco, A. (2004). Low-Dose Balsalazide Plus a Highpotency Probiotic Preparation Is More Effective Than Balsalazide Alone or Mesalazine in the Treatment of Acute Mild-to-Moderate Ulcerative Colitis. *Medical Science Monitor,* Vol.10, No.11, (November 2004), pp. PI126-131, ISSN 1234-1010

Van de Wiele, T., Gallawa, C. M., Kubachka, K. M., Creed, J. T., Basta, N., Dayton, E. A., Whitacre, S., Du Laing, G., & Bradham, K. (2010). Arsenic Metabolism by Human Gut Microbiota Upon in Vitro Digestion of Contaminated Soils. *Environmental Health Perspectives,* Vol.118, No.7, (July 2010), pp. 1004-1009, ISSN 0091-6765

Van de Wiele, T., Vanhaecke, L., Boeckaert, C., Peru, K., Headley, J., Verstraete, W., & Siciliano, S. (2005). Human Colon Microbiota Transform Polycyclic Aromatic Hydrocarbons to Estrogenic Metabolites. *Environmental Health Perspectives,* Vol.113, No.1, (January 2005), pp. 6-10, ISSN 0091-6765

Van den Abbeele, P., Roos, S., Eeckhaut, V., Marzorati, M., Possemiers, S., Vanhoecke, B., Verstraete, W., Van Immerseel, F., & Van de Wiele, T. (2011). Incorporation of a Mucosal Environment in a Dynamic Gut Model Results in a More Representative Colonization by Lactobacilli. "submitted for publication"

Van Gorkom, B. A. P., De Vries, E. G. E., Karrenbeld, A., & Kleibeuker, J. H. (1999). Review Article: Anthranoid Laxatives and Their Potential Carcinogenic Effects. *Alimentary Pharmacology & Therapeutics,* Vol.13, No.4, (April 1999), pp. 443-452, ISSN 0269-2813

van Tassell, R. L., Carman, R. J., Kingston, D. G., & Wilkins, T. D. (1989). Bacterial Metabolism in Humans of the Carcinogen IQ to the Direct Acting Mutagen Hydroxy-IQ. *Microbial Ecology in Health & Disease,* Vol.2, (January 1989), pp. 123-129, ISSN 0891-060X

Vandenbroucke, K., de Haard, H., Beirnaert, E., Dreier, T., Lauwereys, M., Huyck, L., Van Huysse, J., Demetter, P., Steidler, L., Remaut, E., Cuvelier, C., & Rottiers, P. (2010). Orally Administered *L. lactis* Secreting an Anti-TNF Nanobody Demonstrate Efficacy in Chronic Colitis. *Mucosal Immunology,* Vol.3, No.1, (January 2010), pp. 49-56, ISSN 1933-0219

Vandenbroucke, K., Hans, W., Van Huysse, J., Neirynck, S., Demetter, P., Remaut, E., Rottiers, P., & Steidler, L. (2004). Active Delivery of Trefoil Factors by Genetically Modified *Lactococcus lactis* Prevents and Heals Acute Colitis in Mice. *Gastroenterology,* Vol.127, No.2, (August 2004), pp. 502-513, ISSN 0016-5085

Watanabe, T., Kitani, A., Murray, P. J., & Strober, W. (2004). NOD2 Is a Negative Regulator of Toll-Like Receptor 2-Mediated T Helper Type 1 Responses. *Nature Immunology,* Vol.5, No.8, (August 2004), pp. 800-808, ISSN 1529-2908

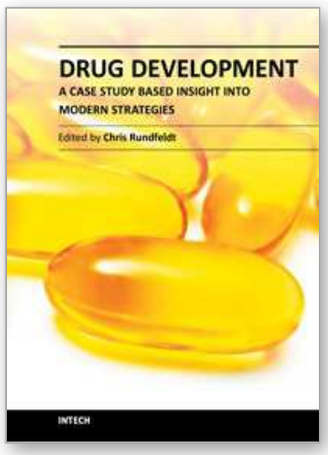
Wenzel, S. C., & Muller, R. (2009). Myxobacteria-'Microbial Factories' for the Production of Bioactive Secondary Metabolites. *Molecular Biosystems,* Vol.5, No.6, (June 2009), pp. 567-574, ISSN 1742-206X

Witztum, J. L. (1994). The Oxidation Hypothesis of Artherosclerosis. *Lancet,* Vol.344, No.8925, (September 1994), pp. 793-795, ISSN 0140-6736

Wu, S. W. P., Pao, C. C., Chan, J., & Yen, T. S. B. (1991). Lack of Mycobacterial DNA in Crohn's Disease Tissue. *Lancet,* Vol.337, No.8734, (January 1991), pp. 174-175, ISSN 0140-6736

Zoetendal, E. G., Akkermans, A. D. L., & De Vos, W. M. (1998). Temperature Gradient Gel Electrophoresis Analysis of 16S rRNA from Human Fecal Samples Reveals Stable and Host-Specific Communities of Active Bacteria. *Applied and Environmental Microbiology,* Vol.64, No.10, (October 1998), pp. 3854-3859, ISSN 0099-2240

Zoetendal, E. G., Ben-Amor, K., Akkermans, A. D. L., Abee, T., & de Vos, W. M. (2001). DNA Isolation Protocols Affect the Detection Limit of PCR Approaches of Bacteria in Samples from the Human Gastrointestinal Tract. *Systematic and Applied Microbiology,* Vol.24, No.3, (November 2001), pp. 405-410, ISSN 0723-2020



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This book represents a case study based overview of many different aspects of drug development, ranging from target identification and characterization to chemical optimization for efficacy and safety, as well as bioproduction of natural products utilizing for example lichen. In the last section, special aspects of the formal drug development process are discussed. Since drug development is a highly complex multidisciplinary process, case studies are an excellent tool to obtain insight in this field. While each chapter gives specific insight and may be read as an independent source of information, the whole book represents a unique collection of different facets giving insight in the complexity of drug development.

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